CD27L ANTIGEN BINDING PROTEINS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/538,024 filed on September 22, 2011, the contents of which are hereby incorporated by reference in their entirety.

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled A-1437-US-NP(US Non-Prov)_ST25.txt, created September 17, 2012, which is 94.3 KB in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The field of this invention relates to compositions of antigen binding proteins including antibodies capable of binding CD27L, as well as related methods.

BACKGROUND

CD27L (CD70, TNFSF7) is a type II integral membrane protein whose expression on normal tissues is highly restricted to a subset of activated T and B cells, dendritic cells and to a small subset of cells in the thymic epithelium. The biological functions of CD27L, which include augmentation or regulation of the immune response, are mediated via binding to its receptor, CD27, which is expressed predominately on lymphoid cells. CD27L/CD27 interactions regulate B-cell proliferation and differentiation and T-cell costimulation/activation. Disruption of the CD27L/CD27 interaction in mice deficient for CD27 does not result in any phenotype in the absence of an immune challenge. (Grewal, *Expert Opin. Ther. Targets.* 12, 341-351 (2008)).

In addition to its very restricted expression on normal tissues, CD27L is expressed at relatively high levels in some B cell non-Hodgkin's lymphoma (B-NHL) tumor sub-types, in pre-B cell acute lymphocytic leukemia (ALL) and in B cell type-chronic lymphocytic leukemia (B-CLL). Aberrant expression of CD27L is also observed in renal cell carcinoma (RCC) but not in normal kidney or other normal tissues. Thus, CD27L comes close to exhibiting properties consistent with those of a "tumor specific antigen" (Grewal, *Expert Opin. Ther. Targets.* 12, 341-351 (2008)).

Each year, of the approximately 49,000 patients that will develop RCC, a little over 40,000 of those will be diagnosed with ccRCC in the US (American Cancer Society: Cancer Facts and Figures final (2008). While some newer therapeutics have been approved for RCC over the last 4 years, the 5 year survival rate for patients with metastatic RCC remains dismal at 10-20% (National Caner Institute. SEER cancer statistics fact sheet: cancer of the kidney and renal pelvis -- accessed 2008) and significant unmet medical need remains. The projected yearly number of newly diagnosed ccRCC patients (U.S.) that are expected to express CD27L is approximately 36,000. There are an estimated 64,000 ccRCC patients currently with active disease.

Of the B-cell malignancies reported to aberrantly express CD27L, the B-NHL subsets of diffuse large cell B-cell lymphoma (DLBCL) and follicular lymphoma (FL) show the highest incidence of expression ranging from 33% for FL to 71% for DLBCL as assessed by IHC on frozen sections using a validated antibody (Lens et al., *Brit. J. Hematol.* 106, 491-503 (1999). 50-89 % of B-CLL tumors also express CD27L as assessed by IHC on frozen tumor sections or by flow cytometry on circulating tumor cells (Ranheim et al., *Blood* 85, 3556-3565 (1965); Trentin et al., *Cancer Res.* 57, 4940-4947 (1997)).

Of the 127,000 patients in the US currently with active B-NHL, approximately 50% of these patients present with the DLBCL (intermediate grade) sub-type (Morton et al., *Blood* 107, 265-276 (2002)). Despite Rituxan plus cyclophosphamide, adriamycin, vincristine, prednisone (CHOP) standard of care therapy for DLBCL patients, almost 50% relapse. Therefore an unmet medical need remains in this disease as well.

SUMMARY

The invention provides anti-CD27L antigen binding proteins, e.g., antibodies and functional fragments thereof. The anti-CD27L antigen binding proteins are particularly useful in methods of treating diseases and disorders associated with aberrant cell proliferation, e.g., cancer, and/or with inflammation.

In a first aspect, the CD27L antigen binding protein comprises a) a light chain variable domain having at least 90% identity, at least 95% identity, or is identical to the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ

ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70; b) a heavy chain variable domain having at least 90% identity, at least 95% identity, or is identical to the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24; or c) the light chain variable domain of a) and the heavy chain variable domain of b).

Preferred antigen binding proteins of the first aspect include those comprising a light chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:63 and a heavy chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:17; those comprising a light chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:64 and a heavy chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:18; those comprising a light chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:65 and a heavy chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:19; those comprising a light chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:66 and a heavy chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:20; those comprising a light chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:67 and a heavy chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:21; those comprising a light chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:68 and a heavy chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:22; those comprising a light chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:69 and a heavy chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:23; and those comprising a light chain variable domain having at least 90%, at least

95%, or is identical to the amino acid sequence set forth in SEQ ID NO:70 and a heavy chain variable domain having at least 90%, at least 95%, or is identical to the amino acid sequence set forth in SEQ ID NO:24.

In a second aspect, the CD27L antigen binding protein comprises a) a light chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70; b) a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24; or c) the light chain variable domain of a) and the heavy chain variable domain of b).

Preferred antigen binding proteins of the second aspect include those comprising a light chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:63 and a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:17; those comprising a light chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:64 and a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:18; those comprising a light chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:65 and a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:19; those comprising a light chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:66 and a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:20; those comprising a light chain variable domain having no

more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:67 and a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:21; those comprising a light chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:68 and a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:22; those comprising a light chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:69 and a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:23; and those comprising a light chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:70 and a heavy chain variable domain having no more than ten or no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:24.

In a third aspect, the CD27L antigen binding protein contains a light chain variable domain comprising a) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:71; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:79; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:87; b) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:72; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:80; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:88; c) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in

SEQ ID NO:73; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:81; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:89; d) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:74; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:82; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:90; e) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:75; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:83; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:91; f) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:76; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:84; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:92; g) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:77; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:85; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:93; or h) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:78; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:86; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:94; and a heavy chain variable domain comprising i) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:25; an HCDR2 having

no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:33; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:41; j) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:26; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:34; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:42; k) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:27; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:35; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:43; l) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:28; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:36; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:44; m) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:29; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:37; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:45; n) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:30; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:38; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:46; o) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:31; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2

sequence set forth in SEQ ID NO:39; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:47; or p) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:32; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:40; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:48.

Preferred CD27L antigen binding proteins of third aspect include those comprising the light chain variable domain of a) and the heavy chain variable domain of i); those comprising the light chain variable domain of b) and the heavy chain variable domain of j); those comprising the light chain variable domain of c) and the heavy chain variable domain of k); those comprising the light chain variable domain of d) and the heavy chain variable domain of l); those comprising the light chain variable domain of e) and the heavy chain variable domain of m); those comprising the light chain variable domain of f) and the heavy chain variable domain of n); those comprising the light chain variable domain of g) and the heavy chain variable domain of o); and those comprising the light chain variable domain of h) and the heavy chain variable domain of p).

In a fourth aspect of the invention, the CD27L antigen binding protein of the first, second, or third aspect binds to human CD27L with an affinity of less than or equal to 2 x 10^{-11} M.

In a fifth aspect of the invention, the CD27L antigen binding protein of the first, second, third, or fourth aspect inhibits binding of CD27L to CD27.

In a sixth aspect of the invention, the CD27L antigen binding protein of the first, second, third, fourth, or fifth aspect is an antibody, such as a human antibody. Preferred antibodies include those antibodies that comprise a light chain having the amino acid sequence set forth in SEQ ID:56 and a heavy chain having the amino acid sequence set forth in SEQ ID NO:10; those that comprise a light chain having the amino acid sequence set forth in SEQ ID:57 and a heavy chain having the amino acid sequence set forth in SEQ ID NO:11; those that comprise a light chain having the amino acid sequence set forth in SEQ ID:58 and a heavy chain having the amino acid sequence set forth in SEQ

ID NO:12; those that comprise a light chain having the amino acid sequence set forth in SEQ ID:59 and a heavy chain having the amino acid sequence set forth in SEQ ID NO:13; those that comprise a light chain having the amino acid sequence set forth in SEQ ID:60 and a heavy chain having the amino acid sequence set forth in SEQ ID NO:14; those that comprise a light chain having the amino acid sequence set forth in SEQ ID:61 and a heavy chain having the amino acid sequence set forth in SEQ ID NO:15; and those that comprise a light chain having the amino acid sequence set forth in SEQ ID:62 and a heavy chain having the amino acid sequence set forth in SEQ ID NO:16.

In a seventh aspect, a CD27L antigen binding protein of the first, second, third, fourth, fifth, or sixth aspect is conjugated to a drug or chemotherapeutic agent. In preferred embodiments, the drug or chemotherapeutic agent is conjugated to the antigen binding protein, e.g. antibody, using a linker. Preferred linkers include non-cleavable linkers such as MCC. A preferred chemotherapeutic agent is DM1. Thus, in particularly preferred embodiments, the seventh aspect provides a CD27L antigen binding protein of the first, second, third, fourth, fifth, or sixth aspect conjugated to DM1 by a MCC linker attached to one or more lysine residues.

The process of conjugating DM1 to a CD27L antigen binding protein, e.g. antibody, will produce a composition comprising a population of DM1-conjugated antibodies having a range of DM1 molecules per antibody. It is possible to measure an average number for the composition. In preferred embodiments, the average number of DM1 molecules per CD27L antigen binding protein, e.g. antibody, is between 1 and 10, between 3 and 7, or between 4 and 6. In preferred embodiments, the composition of CD27L antigen binding proteins of the first, second, third, fourth, fifth, sixth, or seventh aspect of this invention has an average number of DM1 molecules per CD27L antigen binding protein of about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, or about 6.0. Such a composition may contain a therapeutically effective amount of the CD27L antigen binding protein and may be lyophilized.

In an eight aspect, the invention provides isolated nucleic acids encoding one or more polypeptide components of a CD27L antigen binding protein, e.g., an antibody light

chain or antibody heavy chain. In preferred embodiments the nucleic acid encodes a polypeptide comprising:

a) a light chain variable domain having at least 95% identity to the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70;

b) a heavy chain variable domain having at least 95% identity to the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24;

c) a light chain variable domain having no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70;

d) a heavy chain variable domain having no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24;

e) a light chain variable domain comprising:

i) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:71; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:79; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:87;

ii) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:72; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:80; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:88;

iii) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:73; an LCDR2 having

no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:81; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:89;

iv) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:74; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:82; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:90;

v) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:75; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:83; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:91;

vi) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:76; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:84; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:92;

vii) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:77; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:85; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:93; or

viii) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:78; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2

sequence set forth in SEQ ID NO:86; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:94; or

f) a heavy chain variable domain comprising:

i) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:25; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:33; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:41;

ii) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:26; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:34; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:42;

iii) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:27; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:35; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:43;

iv) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:28; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:36; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:44;

v) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:29; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2

sequence set forth in SEQ ID NO:37; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:45;

vi) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:30; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:38; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:46;

vii) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:31; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:39; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:47; or

viii) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:32; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:40; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:48.

In certain embodiments of the eighth aspect, the polypeptide encodes an antibody light chain and is at least 80%, at least 90%, at least 95%, or is 100% identical to the nucleotide sequence set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, or SEQ ID NO:55. In other embodiments of the eighth aspect, the polypeptide encodes an antibody heavy chain and is at least 80%, at least 90%, at least 95%, or is 100% identical to the nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9.

In a ninth aspect, the invention provides an expression vector comprising one or more isolated nucleic acids of the eighth aspect. In certain embodiments, the expression

vector encodes an antibody light chain, an antibody heavy chain, or both an antibody light chain and a heavy chain.

In a tenth aspect, the invention provides a recombinant host cell comprising one or more isolated nucleic acids of the eighth aspect operably linked to a promoter, including recombinant host cells comprising one or more expression vectors of the ninth aspect of the invention. In preferred embodiments, the recombinant host cell secretes an antibody that binds CD27L. Preferred host cells are mammalian host cells, including CHO cell lines.

In an eleventh aspect, the invention provides methods of making a CD27L antibody drug conjugate of the seventh aspect by conjugating a linker and drug, e.g., chemotherapeutic agent, to any of the CD27L antigen binding proteins of the first, second, third, fourth, fifth, or sixth aspects. The linker and drug may be connect first and then conjugated to the CD27L antigen binding protein or the linker may be first conjugated to the CD27L antigen binding protein then connected to the drug. In preferred embodiments, the linker is MCC and the drug is DM1. In particularly preferred embodiments, the CD27L antigen binding protein is an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:63 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:17 (e.g., Ab1), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:64 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:18 (e.g., Ab2), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:65 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:19 (e.g., Ab3), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:66 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:20 (e.g., Ab4), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:67 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:21 (e.g., Ab5), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:68 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:22 (e.g., Ab6), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:69 and a heavy

chain variable domain amino acid sequence as set forth in SEQ ID NO:23 (e.g., Ab7), or an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:70 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:24 (e.g., Ab8) conjugated to DM1 by MCC by chemically reacting one or more lysine residues within the antibody with MCC or MCC-DM1.

In a twelfth aspect, the invention provides methods of treating cancer comprising administering to a patient a therapeutically effective amount of a composition comprising a therapeutically effective amount of a CD27L antigen binding protein of the first, second, third, fourth, fifth, or sixth aspect. In preferred embodiments, the CD27L antigen binding protein is an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:63 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:17 (e.g., Ab1), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:64 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:18 (e.g., Ab2), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:65 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:19 (e.g., Ab3), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:66 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:20 (e.g., Ab4), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:67 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:21 (e.g., Ab5), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:68 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:22 (e.g., Ab6), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:69 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:23 (e.g., Ab7), or an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:70 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:24 (e.g., Ab8). In particularly preferred embodiments, the CD27L antigen binding protein is conjugated to a chemotherapeutic agent (e.g., DM1) by a linker (MCC). In other

preferred embodiments of the twelfth aspect, the antibody comprises enhanced effector function.

In some embodiments, the CD27L antigen binding protein is administered to a patient having renal cell carcinomas (RCC), clear cell RCC, head and neck cancer, glioblastoma, breast cancer, brain tumor, nasopharangeal carcinoma, non-Hodgkin's lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), Burkitt's lymphoma, anaplastic large-cell lymphomas (ALCL), multiple myeloma, cutaneous T-cell lymphomas, nodular small cleaved-cell lymphomas, lymphocytic lymphomas, peripheral T-cell lymphomas, Lennert's lymphomas, immunoblastic lymphoma, T-cell leukemia/lymphomas (ATLL), adult T-cell leukemia (T-ALL), entroblastic/centrocytic (cb/cc) follicular lymphoma cancer, diffuse large cell lymphoma of B lineage, angioimmunoblastic lymphadenopathy (AILD)-like T cell lymphoma, HIV associated body cavity based lymphoma, embryonal carcinoma, undifferentiated carcinoma of the rhino-pharynx, Castleman's disease, Kaposi's Sarcoma, multiple myeloma, Waldenstrom's macroglobulinemia or other B-cell lymphoma.

In certain embodiments, a sample from a patient is tested for CD27L expression prior to administering the CD27L antigen binding protein. CD27L expression may be determined by testing for the presence of CD27L-encoding RNA or for the presence of CD27L protein in the sample. The sample may be a blood sample or biopsy.

In a thirteenth aspect, the invention provides methods of treating an autoimmune or inflammatory disorder said method comprising administering a therapeutically effective amount of a CD27L antigen binding protein of any one of the first, second, third, fourth, fifth, or sixth aspects to a patient in need thereof. In preferred embodiments, the CD27L antigen binding protein is an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:63 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:17 (e.g., Ab1), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:64 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:18 (e.g., Ab2), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:65 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:19 (e.g., Ab3), an antibody comprising a light

chain variable domain amino acid sequence as set forth in SEQ ID NO:66 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:20 (e.g., Ab4), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:67 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:21 (e.g., Ab5), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:68 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:22 (e.g., Ab6), an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:69 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:23 (e.g., Ab7), or an antibody comprising a light chain variable domain amino acid sequence as set forth in SEQ ID NO:70 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:24 (e.g., Ab8). In preferred embodiments, the CD27L antigen binding protein inhibits binding of CD27 to CD27L. In particularly preferred embodiments, the autoimmune or inflammatory disorder is systemic lupus erythematosus (SLE), insulin dependent diabetes mellitus (IDDM), inflammatory bowel disease (IBD), multiple sclerosis (MS), psoriasis, autoimmune thyroiditis, rheumatoid arthritis (RA), or glomerulonephritis. In other embodiments, treatment inhibits or prevents transplant rejection or graft versus host disease in the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Summary of functional and physical characteristics of exemplary embodiments of CD27L antigen binding proteins.

FIG. 2. Measurement of the level and the rate of internalization of Ab4 (A4) and Ab8 (A8) and their conjugated counterparts into 786-0 cells over time (A).

FIG. 3. 786-0 cells were cultured for 4 days in the presence of increasing concentrations of anti streptavidin-MCC-DM1, Ab4-MCC-DM1 and Ab8-MCC-DM1. Effect on cell growth was measured using the CELL-TITER-GLO reagent that measures cell number using a luminescent read-out.

FIG. 4. Dose response of Ab4-MCC-DM1 and Ab8-MCC-DM1 in the 786-0 xenograft model. Intravenous dose on day 24 is denoted by arrow at the indicated doses.

FIG. 5. Dose response of Ab4-MCC-DM1 and Ab8-MCC-DM1 in the Caki-1 xenograft model. Intravenous dosing schedule is denoted by the arrows.

FIG. 6. Dose response of Ab4-MCC-DM1 in the Raji xenograft model. The intravenous dosing schedule is denoted by the arrows.

FIG. 7. Variable dose response of Ab4-MCC-DM1 in the 786-0 xenograft model.

FIG. 8. Ab4-MCC-DM1 Antibody Dependent Cell Cytotoxicity (ADCC) Assay against Raji tumor cells.

FIG. 9. Ab4-MCC-DM1 Antibody Dependent Cellular Phagocytosis (ADCP) Assay against both Raji and 786-0 tumor cells.

FIG. 10. Comparison of CD27L expression for primary frozen tumor samples that scored "positive" by masked IHC. The results indicate that overall CD27L protein expression is highest in ccRCC patient samples, followed by DLBCL (Diffuse Large B-Cell Lymphoma); B-CLL and FL samples.

FIG. 11. Results of CD27L mRNA and Protein Expression Analysis. The results indicate that there is a high prevalence of CD27L expression in RCC, B-NHL and CLL cells.

FIG. 12. Sets forth the structure of the Ab-MCC-DM1 ADCs described in Example 2 herein.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited within the body of this specification are expressly incorporated by reference in their entirety.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, tissue culture and transformation, protein purification, etc. Enzymatic reactions and purification techniques may be performed according to the manufacturer's specifications or as commonly accomplished in the art or as described herein. The following procedures and techniques may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the specification. *See, e.g.,* Sambrook *et al.*, 2001, *Molecular Cloning: A Laboratory Manuel*, 3rd ed., Cold Spring Harbor Laboratory Press, cold Spring Harbor, N.Y., which is incorporated herein by

reference for any purpose. Unless specific definitions are provided, the nomenclature used in connection with, and the laboratory procedures and techniques of, analytic chemistry, organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, chemical analyses, pharmaceutical preparation, formulation, and delivery and treatment of patients.

CD27L

The antigen binding proteins bind to CD27L, which is also known as CD70 and TNFSF7. CD27L was first described in US Pat. No. 5,573,924. An exemplary human CD27L amino acid sequence is provided herein as SEQ ID NO:1, which corresponds to NCBI Reference Sequence NP_001423.1 (GI:4507605). In certain embodiments, the antigen binding protein blocks the interaction of CD27L with its receptor CD27. An exemplary CD27 amino acid sequence is provided as SEQ ID NO:2, which corresponds to Swiss-Prot: P26842.2 (GI:269849546). A mature CD27 amino acid sequence corresponds to amino acids 20-260 of SEQ ID NO:2.

CD27L Antigen Binding Proteins

The present invention provides antigen binding proteins that specifically bind CD27L. Embodiments of antigen binding proteins comprise peptides and/or polypeptides that specifically bind CD27L. Such peptides or polypeptides may optionally include one or more port-translational modification. Embodiments of antigen binding proteins comprise antibodies and fragments thereof, as variously defined herein, that specifically bind CD27L. These include antibodies that specifically bind human CD27L, including those that inhibit CD27L from binding and/or activating CD27.

The antigen binding proteins of the invention specifically bind to CD27L. "Specifically binds" as used herein means that the antigen binding protein preferentially binds CD27L over other proteins. In some embodiments "specifically binds" means the CD27L antigen binding protein has a higher affinity for CD27L than for other proteins. CD27L antigen binding proteins that specifically bind CD27L may have a binding affinity for human CD27L of less than or equal to 1×10^{-7} M, less than or equal to 2×10^{-7} M, less than or equal to 3×10^{-7} M, less than or equal to 4×10^{-7} M, less than or equal to 5×10^{-7} M, less than or equal to 6×10^{-7} M, less than or equal to 7×10^{-7} M, less than or

equal to 8 x 10^{-7} M, less than or equal to 9 x 10^{-7} M, less than or equal to 1 x 10^{-8} M, less than or equal to 2 x 10^{-8} M, less than or equal to 3 x 10^{-8} M, less than or equal to 4 x 10^{-8} M, less than or equal to 5 x 10^{-8} M, less than or equal to 6 x 10^{-8} M, less than or equal to 7 x 10^{-8} M, less than or equal to 8 x 10^{-8} M, less than or equal to 9 x 10^{-8} M, less than or equal to 1 x 10^{-9} M, less than or equal to 2 x 10^{-9} M, less than or equal to 3 x 10^{-9} M, less than or equal to 4 x 10^{-9} M, less than or equal to 5 x 10^{-9} M, less than or equal to 6 x 10^{-9} M, less than or equal to 7 x 10^{-9} M, less than or equal to 8 x 10^{-9} M, less than or equal to 9 x 10^{-9} M, less than or equal to 1 x 10^{-10} M, less than or equal to 2 x 10^{-10} M, less than or equal to 3 x 10^{-10} M, less than or equal to 4 x 10^{-10} M, less than or equal to 5 x 10^{-10} M, less than or equal to 6 x 10^{-10} M, less than or equal to 7 x 10^{-10} M, less than or equal to 8 x 10^{-10} M, less than or equal to 9 x 10^{-10} M, less than or equal to 1 x 10^{-11} M, less than or equal to 2 x 10^{-11} M, less than or equal to 3 x 10^{-11} M, less than or equal to 4 x 10^{-11} M, less than or equal to 5 x 10^{-11} M, less than or equal to 6 x 10^{-11} M, less than or equal to 7 x 10^{-11} M, less than or equal to 8 x 10^{-11} M, less than or equal to 9 x 10^{-11} M, less than or equal to 1 x 10^{-12} M, less than or equal to 2 x 10^{-12} M, less than or equal to 3 x 10^{-12} M, less than or equal to 4 x 10^{-12} M, less than or equal to 5 x 10^{-12} M, less than or equal to 6 x 10^{-12} M, less than or equal to 7 x 10^{-12} M, less than or equal to 8 x 10^{-12} M, or less than or equal to 9 x 10^{-12} M. Methods of measuring the binding affinity of an antigen binding protein are well known in the art. Example 1 provides an exemplary method.

It is understood that when reference is made to the various embodiments of the CD27L-binding antibodies herein, that it also encompasses CD27L-binding fragments thereof. A CD27L-binding fragment comprises any of the antibody fragments or domains described herein that retains the ability to specifically bind to CD27L. The CD27L-binding fragment may be in any of the scaffolds described herein.

In certain therapeutic embodiments, a CD27L antigen binding protein inhibits binding of CD27L to CD27 and/or inhibits one or more biological activities associated with the binding of CD27L to CD27, e.g., CD27-mediated signaling. Such antigen binding proteins are said to be "neutralizing." In certain embodiments, the neutralizing CD27L antigen binding protein specifically binds CD27L and inhibits binding of CD27L to CD27 from anywhere between 10% to 100%, such as by at least about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47,

48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or more. For example, CD27L antigen binding proteins may be tested for neutralizing ability by determining the ability of the antigen binding protein to block binding of CD27-Fc to MP-1 cells (Slack et al, Int. Immunol. (1995) 7(7): 1087-1092)).

Embodiments of antigen binding proteins comprise a scaffold structure, as variously defined herein, with one or more complementarity determining regions (CDRs). Embodiments further include antigen binding proteins comprising a scaffold structure with one or more antibody variable domains, either heavy or light. Embodiments include antibodies that comprise a light chain variable domain selected from the group consisting of Ab1 Light Chain Variable Domain (LCv), Ab2 LCv, Ab3 LCv, Ab4 LCv, Ab5 LCv, Ab6 LCv, Ab7 LCv, and Ab8 LCv (SEQ ID NO:63-70, respectively) and/or a heavy chain variable domain selected from the group consisting of Ab1 Heavy Chain Variable Domain (HCv), Ab2 HCv, Ab3 HCv, Ab4 HCv, Ab5 HCv, Ab6 HCv, Ab7 HCv, and Ab8 HCv (SEQ ID NO:17-24, respectively), and fragments, derivatives, muteins, and variants thereof.

An exemplary light chain comprising Ab1 LCv is a light chain comprising the amino acid sequence set forth in SEQ ID NO:56.

An exemplary light chain comprising Ab2 LCv is a light chain comprising the amino acid sequence set forth in SEQ ID NO:57.

An exemplary light chain comprising Ab4 LCv is a light chain comprising the amino acid sequence set forth in SEQ ID NO:58.

An exemplary light chain comprising Ab5 LCv is a light chain comprising the amino acid sequence set forth in SEQ ID NO:59.

An exemplary light chain comprising Ab6 LCv is a light chain comprising the amino acid sequence set forth in SEQ ID NO:60.

An exemplary light chain comprising Ab7 LCv is a light chain comprising the amino acid sequence set forth in SEQ ID NO:61.

An exemplary light chain comprising Ab8 LCv is a light chain comprising the amino acid sequence set forth in SEQ ID NO:62.

An exemplary heavy chain comprising Ab1 HCv is a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:10.

An exemplary heavy chain comprising Ab2 HCv is a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:11.

An exemplary heavy chain comprising Ab4 HCv is a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:12.

An exemplary heavy chain comprising Ab5 HCv is a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:13.

An exemplary heavy chain comprising Ab6 HCv is a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:14.

An exemplary heavy chain comprising Ab7 HCv is a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:15.

An exemplary heavy chain comprising Ab8 HCv is a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:16.

Additional examples of scaffolds that are envisioned include: fibronectin, neocarzinostatin CBM4-2, lipocalins, T-cell receptor, protein -A domain (protein Z), Im9, TPR proteins, zinc finger domains, pVIII, avian pancreatic polypeptide, GCN4, WW domain Src homology domain 3, PDZ domains, TEM-1 beta-lactamase, thioredoxin, staphylococcal nuclease, PHD-finger domains, CL-2, BPTI, APPI, HPSTI, ecotin, LACI-D1, LDTI, MTI-II, scorpion toxins, insect defensin-A peptide, EETI-II, Min-23, CBD, PBP, cytochrome b-562, Ldl receptor domains, gamma-crystallin, ubiquitin, transferrin, and or C-type lectin-like domains. Non-antibody scaffolds and their use as therapeutics are reviewed in Gebauer and Skerra, *Curr. Opin. Chem. Biol.*, 13:245-255 (2009) and Binz et al., Nat. Biotech., 23(10):1257-1268 (2005), which are incorporated herein by reference in its entirety.

Aspects of the invention include antibodies comprising the following variable domains: Ab1 LCv/Ab1 HCv (SEQ ID NO:63/SEQ ID NO:17), Ab2 LCv/Ab2 HCv (SEQ ID NO:64/SEQ ID NO:18), Ab3 LCv/Ab3 HCv (SEQ ID NO:65/SEQ ID NO:19), Ab4 LCv/Ab4 HCv (SEQ ID NO:66/SEQ ID NO:20), Ab5 LCv/Ab5 HCv (SEQ ID NO:67/SEQ ID NO:21), Ab6 LCv/Ab6 HCv (SEQ ID NO:68/SEQ ID NO:22), Ab7 LCv/Ab7 HCv (SEQ ID NO:69/SEQ ID NO:23), Ab8 LCv/Ab8 HCv (SEQ ID

NO:70/SEQ ID NO:24), and combinations thereof, as well as fragments, derivatives, muteins and variants thereof.

Exemplary antibodies of the invention include Ab1 (SEQ ID NO:56/SEQ ID NO:10), Ab2 (SEQ ID NO:57/SEQ ID NO:11), Ab4 (SEQ ID NO:58/SEQ ID NO:12), Ab5 (SEQ ID NO:59/SEQ ID NO:13), Ab6 (SEQ ID NO:60/SEQ ID NO:14), Ab7 (SEQ ID NO:61/SEQ ID NO:15), Ab8 (SEQ ID NO:62/SEQ ID NO:16).

Typically, each variable domain of an antibody light or heavy chain comprises three CDRs. The heavy chain variable domain comprises a heavy chain CDR1 (HCDR1), a heavy chain CDR2 (HCDR2), and a heavy chain CDR3 (HCDR3). The light chain variable domain comprises a light chain CDR1 (LCDR1), a light chain CDR2 (LCDR2), and a light chain CDR3 (LCDR3). In certain embodiments, an antigen binding protein comprises one or more CDRs contained within the preferred variable domains described herein.

Examples of such CDRs include, but are not limited to:

the CDRs of Ab1 LCv: LCDR1 (SEQ ID NO:71), LCDR2 (SEQ ID NO:79), and LCDR3 (SEQ ID NO:87);

the CDRs of Ab2 LCv: LCDR1 (SEQ ID NO:72), LCDR2 (SEQ ID NO:80), and LCDR3 (SEQ ID NO:88);

the CDRs of Ab3 LCv: LCDR1 (SEQ ID NO:73), LCDR2 (SEQ ID NO:81), and LCDR3 (SEQ ID NO:89);

the CDRs of Ab4 LCv: LCDR1 (SEQ ID NO:74), LCDR2 (SEQ ID NO:82), and LCDR3 (SEQ ID NO:90);

the CDRs of Ab5 LCv: LCDR1 (SEQ ID NO:75), LCDR2 (SEQ ID NO:83), and LCDR3 (SEQ ID NO:91);

the CDRs of Ab6 LCv: LCDR1 (SEQ ID NO:76), LCDR2 (SEQ ID NO:84), and LCDR3 (SEQ ID NO:92);

the CDRs of Ab7 LCv: LCDR1 (SEQ ID NO:77), LCDR2 (SEQ ID NO:85), and LCDR3 (SEQ ID NO:93);

the CDRs of Ab8 LCv: LCDR1 (SEQ ID NO:78), LCDR2 (SEQ ID NO:86), and LCDR3 (SEQ ID NO:94);

the CDRs of Ab1 HCv: HCDR1 (SEQ ID NO:25), HCDR2 (SEQ ID NO:33), and HCDR3 (SEQ ID NO:41);

the CDRs of Ab2 HCv: HCDR1 (SEQ ID NO:26), HCDR2 (SEQ ID NO:34), and HCDR3 (SEQ ID NO:42);

the CDRs of Ab3 HCv: HCDR1 (SEQ ID NO:27), HCDR2 (SEQ ID NO:35), and HCDR3 (SEQ ID NO:43);

the CDRs of Ab4 HCv: HCDR1 (SEQ ID NO:28), HCDR2 (SEQ ID NO:36), and HCDR3 (SEQ ID NO:44);

the CDRs of Ab5 HCv: HCDR1 (SEQ ID NO:29), HCDR2 (SEQ ID NO:37), and HCDR3 (SEQ ID NO:45);

the CDRs of Ab6 HCv: HCDR1 (SEQ ID NO:30), HCDR2 (SEQ ID NO:38), and HCDR3 (SEQ ID NO:46);

the CDRs of Ab7 HCv: HCDR1 (SEQ ID NO:31), HCDR2 (SEQ ID NO:39), and HCDR3 (SEQ ID NO:47); and

the CDRs of Ab8 HCv: HCDR1 (SEQ ID NO:32), HCDR2 (SEQ ID NO:40), and HCDR3 (SEQ ID NO:48).

In some embodiments, the antigen binding protein comprises: A) a polypeptide, e.g., a light chain, that comprises an LCDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:71, 72, 73, 74, 75, 76, 77, and 78; an LCDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 79, 80, 81, 82, 83, 84, 85, and 86; and/or an LCDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:87, 88, 89, 90, 91, 92, 93, and 94; and/or B) a polypeptide, e.g., a heavy chain, that comprises an HCDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS:25, 26, 27, 28, 29, 30, 31, and 32; an HCDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS:33, 34, 35, 36, 37, 38, 39, and 40; and/or an HCDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS:41, 42, 43, 44, 45, 46, 47, and 48.

In further embodiments, the antigen binding protein comprise A) a light chain amino acid sequence that comprises a LCDR1, LCDR2, and LCDR3 of any of Ab1 LCv, Ab2 LCv, Ab3 LCv, Ab4 LCv, Ab5 LCv, Ab6 LCv, Ab7 LCv, and Ab8 LCv, and B) a

heavy chain amino acid sequence that comprises a HCDR1, HCDR2, and HCDR3 of any of Ab1 HCv, Ab2 HCv, Ab3 HCv, Ab4 HCv, Ab5 HCv, Ab6 HCv, Ab7 HCv, and Ab8 HCv.

In certain embodiments, the CDRs include no more than one, no more than two, no more than three, no more than four, no more than five, or no more than six amino acid additions, deletions, or substitutions from an exemplary CDR set forth herein.

Aspects of the invention include antibodies comprising a light chain variable domain selected from the group consisting of SEQ ID NOS:63, 64, 65, 66, 67, 68, 69, and 70. Aspects of the invention include antibodies comprising a heavy chain variable domain selected from the group consisting of SEQ ID NOS:17, 18, 19, 20, 21, 22, 23, and 24. Further aspects of the invention include antibodies comprising A) a light chain variable domain selected from the group consisting of SEQ ID NOS:63, 64, 65, 66, 67, 68, 69, and 70, and B) a heavy chain variable domain selected from the group consisting of SEQ ID NOS:17, 18, 19, 20, 21, 22, 23, and 24.

Antibodies of the invention can comprise any constant region known in the art. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, e.g., a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region, e.g., a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. In one embodiment the light or heavy chain constant region is a fragment, derivative, variant, or mutein of a naturally occurring constant region.

Aspects of the invention include antibodies comprising a light chain variable region selected from the group consisting of SEQ ID NOS:63, 64, 65, 66, 67, 68, 69, and 70 having no more than one, no more than two, no more than three, no more than four, no more than five, no more than six, no more than seven, no more than eight, no more than nine, or no more than ten amino acid additions, deletions, or substitutions. Aspects of the invention include antibodies comprising a heavy chain variable region selected from the group consisting of SEQ ID NOS:17, 18, 19, 20, 21, 22, 23, and 24 having no more than one, no more than two, no more than three, no more than four, no more than five, no more than six, no more than seven, no more than eight, no more than nine, or no more

than ten amino acid additions, deletions, or substitutions. Further aspects of the invention include antibodies comprising A) comprising a light chain variable region selected from the group consisting of SEQ ID NOS:63, 64, 65, 66, 67, 68, 69, and 70 having no more than one, no more than two, no more than three, no more than four, no more than five, no more than six, no more than seven, no more than eight, no more than nine, or no more than ten amino acid additions, deletions, or substitutions, and B)a heavy chain variable region selected from the group consisting of SEQ ID NOS:17, 18, 19, 20, 21, 22, 23, and 24 having no more than one, no more than two, no more than three, no more than four, no more than five, no more than six, no more than seven, no more than eight, no more than nine, or no more than ten amino acid additions, deletions, or substitutions. For example, in certain exemplary embodiments, an antibody comprises 1) a variant of the light chain variable domain set forth in SEQ ID NO:66, wherein the phenylalanine at position 51 is mutated to a leucine and/or the proline at position 105 is mutated to a glycine or a glutamine; 2) a variant of the heavy chain variable domain set forth in SEQ ID NO:20, wherein the glutamine at position 1 is mutated to glutamic acid and/or the arginine at position 16 is mutated to a glycine; or a variant of the light chain variable domain set forth in SEQ ID NO:66, wherein the phenylalanine at position 51 is mutated to a leucine and/or the proline at position 105 is mutated to a glycine or a glutamine and a variant of the heavy chain variable domain set forth in SEQ ID NO:20, wherein the glutamine at position 1 is mutated to glutamic acid and/or the arginine at position 16 is mutated to a glycine.

In one variation, the antigen binding protein comprises an amino acid sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a light chain amino acid sequence selected from the group consisting of SEQ ID NOS:63, 64, 65, 66, 67, 68, 69, and 70. In another variation, the antigen binding protein comprises an amino acid sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a

heavy chain amino acid sequence selected from the group consisting of SEQ ID NOS:17, 18, 19, 20, 21, 22, 23, and 24. In yet a further embodiment, the antigen binding protein comprises A) an amino acid sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a light chain amino acid sequence selected from the group consisting of SEQ ID NOS:63, 64, 65, 66, 67, 68, 69, and 70, and B) an amino acid sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOS:17, 18, 19, 20, 21, 22, 23, and 24.

In certain embodiments, the antigen binding protein comprises a light chain and/or heavy chain CDR3. In some embodiments, the antigen binding protein comprises an amino acid sequence selected from the group of sequences set forth in SEQ ID NOS:87, 88, 89, 90, 91,92, 93,94, 41, 42, 43, 44, 45, 46, 47, and 48. In certain embodiments, the amino acid sequence includes no more than one, no more than two, no more than three, no more than four, no more than five, or no more than six amino acid additions, deletions, or substitutions from the exemplary sequence set forth in SEQ ID NOS:87, 88, 89, 90, 91,92, 93,94, 41, 42, 43, 44, 45, 46, 47, and 48.. Thus, embodiments of the invention include antigen binding protein comprising an amino acid sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence selected from the group of sequences set forth in SEQ ID NOS:87, 88, 89, 90, 91,92, 93,94, 41, 42, 43, 44, 45, 46, 47, and 48.

In certain embodiments, the antigen binding protein comprises a light chain and/or heavy chain CDR2. In some embodiments, the antigen binding protein comprises an amino acid sequence selected from the group of sequences set forth in SEQ ID NOS:79, 80, 81, 82, 83, 84, 85, 86, 33, 34, 35, 36, 37, 38, 39, and 40. In certain

embodiments, the amino acid sequence includes no more than one, no more than two, no more than three, no more than four, no more than five, or no more than six amino acid additions, deletions, or substitutions from the exemplary sequence set forth in SEQ ID NOS: 79, 80, 81, 82, 83, 84, 85, 86, 33, 34, 35, 36, 37, 38, 39, and 40.. Thus, embodiments of the invention include antigen binding protein comprising an amino acid sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence selected from the group of sequences set forth in SEQ ID NOS: 79, 80, 81, 82, 83, 84, 85, 86, 33, 34, 35, 36, 37, 38, 39, and 40.

In certain embodiments, the antigen binding protein comprises a light chain and/or heavy chain CDR1. In some embodiments, the antigen binding protein comprises an amino acid sequence selected from the group of sequences set forth in SEQ ID NOS:71, 72, 73, 74, 75, 76, 77, 78, 25, 26, 27, 28, 29, 30, 31, and 32. In certain embodiments, the amino acid sequence includes no more than one, no more than two, no more than three, no more than four, no more than five, or no more than six amino acid additions, deletions, or substitutions from the exemplary sequence set forth in SEQ ID NOS: 71, 72, 73, 74, 75, 76, 77, 78, 25, 26, 27, 28, 29, 30, 31, and 32.. Thus, embodiments of the invention include antigen binding protein comprising an amino acid sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to an amino acid sequence selected from the group of sequences set forth in SEQ ID NOS: 71, 72, 73, 74, 75, 76, 77, 78, 25, 26, 27, 28, 29, 30, 31, and 32.

The antigen binding proteins of the invention comprise the scaffolds of traditional antibodies, including human and monoclonal antibodies, bispecific antibodies, diabodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively. The above described CDRs,

including various combinations of the CDRs, may be grafted into any of the following scaffolds.

As used herein, the term "antibody" refers to the various forms of monomeric or multimeric proteins comprising one or more polypeptide chains that specifically binds to an antigen, as variously described herein. In certain embodiments, antibodies are produced by recombinant DNA techniques. In additional embodiments, antibodies are produced by enzymatic or chemical cleavage of naturally occurring antibodies. In another aspect, the antibody is selected from the group consisting of: a) a human antibody; b) a humanized antibody; c) a chimeric antibody; d) a monoclonal antibody; e) a polyclonal antibody; f) a recombinant antibody; g) an antigen-binding fragment; h) a single chain antibody; i) a diabody; j) a triabody, k) a tetrabody, l) a Fab fragment; m) a $F(ab')_2$ fragment, n) an IgA antibody, o) an IgD antibody, p) an IgE antibody, q) an IgG1 antibody, r) an IgG2 antibody, s) an IgG3 antibody, t) an IgG4 antibody, and u) an IgM antibody.

A variable region comprises at least three heavy or light chain CDRs embedded within a framework region (designated framework regions FR1, FR2, FR3, and FR4). Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest*, Public Health Service N.I.H., Bethesda, MD. Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" and one "heavy" chain. The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa or lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to IgM1 and IgM2. Embodiments of the invention include all such classes and subclasses of antibodies that incorporate a variable domain or CDR of the antigen binding proteins, as described herein.

Some naturally occurring antibodies, such as those found in camels and llamas, are dimers consisting of two heavy chains and include no light chains. The invention encompasses dimeric antibodies of two heavy chains, or fragments thereof, that can bind to CD27L.

The variable regions of the heavy and light chains typically exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, i.e., the complementarity determining regions or CDRs. The CDRs are primarily responsible for antigen recognition and binding. The CDRs fromt eh two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat.

CDRs constitute the major surface contact points for antigen binding. The CDR3 or the light chain and, particularly, CDR3 of the heavy chain may constitute the most important determinants in antigen binding within the light and heavy chain variable regions. In some antibodies, the heavy chain CDR3 appears to constitute the major area of contact between the antigen and the antibody. In vitro selection schemes in which CDR3 alone is varied can be used to vary the binding properties of an antibody or determine which residues contribute to the binding of an antigen.

Naturally occurring antibodies typically include a signal sequence, which directs the antibody into the cellular pathway for protein secretion and which is typically not present in the mature antibody. A polynucleotide encoding an antibody of the invention may encode a naturally occurring a signal sequence or a heterologous signal sequence as described below.

In one embodiment, the antigen binding protein is a antibody comprising from one to six of the exemplary CDRs described herein. The antibodies of the invention may be of any type including IgM, IgG (including IgG1, IgG2, IgG3, IgG4), IgD, IgA, or IgE antibody. In a specific embodiment the antigen biding protein is an IgG type antibody, e.g., a IgG1 antibody.

In some embodiments, for example when the antigen binding protein is an antibody with complete heavy and light chains, the CDRs are all from the same species,

e.g., human. Alternatively, for example in embodiments wherein the antigen binding protein contains less than six CDRs from the sequences outlined above, additional CDRs may be either from other species or may be different human CDRs than those depicted in the exemplary sequences. For example, HCDR3 and LCDR3 regions from the appropriate sequences identified herein may be used with HCDR1, HCDR2, LCDR1, and LCDR2 being optionally selected from alternate species or different human antibody sequences, or combinations thereof. For example, the CDRs of the invention can replace the CDR regions of commercially relevant chimeric or humanized antibodies.

Specific embodiments utilize scaffold components of the antigen binding proteins that are human components. In some embodiments, however, the scaffold components can be a mixture from different species. As such, if the antigen binding protein is an antibody, such antibody may be a chimeric antibody and/or humanized antibody. In general, both "chimeric antibodies" and humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some casaes) and the constant region(s) from a human.

"Humanized antibodies" generally refer to non-human antibodies that have had the variable domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except one or more CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within one or more CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, *e.g.*, WO 92/11018, Jones 1986, *Nature* 321:522-525, Verhoeyen *et al.*, 1988, *Science* 239:1534-1536. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654. In the exemplary embodiments described herein, the identified CDRs are human, and thus both humanized and chimeric antibodies in this context include some non-human CDRs; for example, humanized antibodies may be generated that comprise the

HCDR3 and LCDR3 regions, with one or more of the other CDR regions being of a different species origin.

In one embodiment, theCD27L antigen binding protein is a mutlispecific antibody, and notably a bispecfic antibody, also sometimes referred to as "diabodies." These are antibodies that bind to two or more different antigens or different epitopes on a single antigen. In certain embodiments, a bispecific antibody binds CD27L and an antigen on a human effector cell (e.g., T cell). Such antibodies are useful in targeting an effector cell response against a CD27L expressing cells, such as a tumor cell. In preferred embodiments, the human effector cell antigen is CD3. U.S. Pat. No. 7,235,641. Methods of making bispecific antibodies are known in the art. One such method involves engineering the Fc portion of the heavy chains such as to create "knobs" and "holes" which facilitate heterodimer formation of the heavy chains when co-expressed in a cell. U.S. 7,695,963. Another method also involves engineering the Fc portion of the heavy chain but uses electrostatic steering to encourage heterodimer formation while discouraging homodimer formation of the heavy chains when co-expressed in a cell. WO 09/089,004, which is incorporated herein by reference in its entirety.

In one embodiment, the CD27L antigen binding protein is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. Hu *et al.*, 1996, *Cancer Res.* 56:3055-3061.

In one embodiment, the CD27L antigen binding protein is a domain antibody; *see,* for example U.S. Patent No. 6,248,516. Domain antibodies (dAbs) are functional binding domains of antibodies, corresponding to the variable regions of either the heavy (VH) or light (VL) chains of human antibodies. dABs have a molecular weight of approximately 13 kDa, or less than one-tenth the size of a full antibody. dABs are well expressed in a variety of hosts including bacterial, yeast, and mammalian cell systems. In addition, dAbs are highly stable and retain activity even after being subjected to harsh conditions, such as freeze-drying or heat denaturation. *See*, for example, US Patent 6,291,158; 6,582,915; 6,593,081; 6,172,197; US Serial No. 2004/0110941; European Patent 0368684; US Patent 6,696,245, WO04/058821, WO04/003019 and WO03/002609.

In one embodiment, the CD27L antigen binding protein is an antibody fragment, that is a fragment of any of the antibodies outlined herein that retain binding specificity to

CD27L. In various embodiments, the antibody binding proteins comprise, but are not limited to, a F(ab), F(ab'), F(ab')2, Fv, or a single chain Fv fragments. At a minimum, an antibody, as meant herein, comprises a polypeptide that can bind specifically to CD27L comprising all or part of a light or heavy chain variable region, such as one or more CDRs.

Further examples of CD27L-binding antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward *et al.*, 1989, *Nature* 341:544-546) which consists of a single variable, (v) isolated CDR regions, (vi) $F(ab')_2$ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird *et al.*, 1988, *Science* 242:423-426, Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:5879-5883), (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion (Tomlinson *et. al.*, 2000, *Methods Enzymol.* 326:461-479; WO94/13804; Holliger *et al.*, 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90:6444-6448). The antibody fragments may be modified. For example, the molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains (Reiter *et al.*, 1996, *Nature Biotech.* 14:1239-1245). Aspects of the invention include embodiments wherein the non-CDR components of these fragments are human sequences.

In one embodiment, the CD27L antigen binding protein is a fully human antibody. In this embodiment, as outlined above, specific structures comprise complete heavy and light chains depicted comprising the CDR regions. Additional embodiments utilize one or more of the CDRs of the invention, with the other CDRs, framework regions, J and D regions, constant regions, etc., coming from other human antibodies. For example, the CDRs of the invention can replace the CDRs of any number of human antibodies, particularly commercially relevant antibodies

Single chain antibodies may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in

a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (V_L and V_H). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (*e.g.*, dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt *et al.*, 1997, Prot. Eng. 10:423; Kortt *et al.*, 2001, Biomol. Eng. 18:95-108). By combining different V_L and V_H-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum *et al.*, 2001, Biomol. Eng. 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Patent No. 4,946,778; Bird, 1988, Science 242:423; Huston *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:5879; Ward *et al.*, 1989, Nature 334:544, de Graaf *et al.*, 2002, Methods Mol Biol. 178:379-87. Single chain antibodies derived from antibodies provided herein (including but not limited to scFvs comprising the variable domain combinations of Ab1 LCv/Ab1 HCv (SEQ ID NO:63/SEQ ID NO:17), Ab2 LCv/Ab2 HCv (SEQ ID NO:64/SEQ ID NO:18), Ab3 LCv/Ab3 HCv (SEQ ID NO:65/SEQ ID NO:19), Ab4 LCv/Ab4 HCv (SEQ ID NO:66/SEQ ID NO:20), Ab5 LCv/Ab5 HCv (SEQ ID NO:67/SEQ ID NO:21), Ab6 LCv/Ab6 HCv (SEQ ID NO:68/SEQ ID NO:22), Ab7 LCv/Ab7 HCv (SEQ ID NO:69/SEQ ID NO:23), Ab8 LCv/Ab8 HCv (SEQ ID NO:70/SEQ ID NO:24), and combinations thereof are encompassed by the present invention.

In one embodiment, the CD27L antigen binding protein is an antibody fusion protein (sometimes referred to herein as an "antibody conjugate"). The conjugate partner can be proteinaceous or non-proteinaceous; the latter generally being generated using functional groups on the antigen binding protein and on the conjugate partner. In certain embodiments, the antibody is conjugated to a non-proteinaceous chemical (drug) to form an antibody drug conjugate. Exemplary antibody drug conjugates and methods of making such conjugates are discussed below.

In one embodiment, the CD27L antigen binding protein is an antibody analog, sometimes referred to as "synthetic antibodies." For example, a variety of work utilizes either alternative protein scaffolds or artificial scaffolds with grafted CDRs. Such scaffolds include, but are not limited to, mutations introduced to stabilize the three-

dimensional structure of the binding protein as well as wholly synthetic scaffolds consisting for example of biocompatible polymers. *See*, for example, Korndorfer *et al.*, 2003, *Proteins: Structure, Function, and Bioinformatics*, Volume 53, Issue 1:121-129. Roque *et al.*, 2004, *Biotechnol. Prog.* 20:639-654. In addition, peptide antibody mimetics ("PAMs") can be used, as well as work based on antibody mimetics utilizing fibronection components as a scaffold.

By "protein," as used herein, is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. In some embodiments, the two or more covalently attached amino acids are attached by a peptide bond. The protein may be made up of naturally occurring amino acids and peptide bonds, for example when the protein is made recombinantly using expression systems and host cells, as outlined below. Alternatively, the protein may include synthetic amino acids (*e.g.*, homophenylalanine, citrulline, ornithine, and norleucine), or peptidomimetic structures, *i.e.*, "peptide or protein analogs", such as peptoids (*see*, Simon *et al.*, 1992, *Proc. Natl. Acad. Sci. U.S.A.* 89:9367, incorporated by reference herein), which can be resistant to proteases or other physiological and/or storage conditions. Such synthetic amino acids may be incorporated in particular when the antigen binding protein is synthesized *in vitro* by conventional methods well known in the art. In addition, any combination of peptidomimetic, synthetic and naturally occurring residues/structures can be used. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. The amino acid "R group" or "side chain" may be in either the (L)- or the (S)-configuration. In a specific embodiment, the amino acids are in the (L)- or (S)-configuration.

In certain aspects, the invention provides recombinant antigen binding proteins that bind a CD27L and, in some embodiments, a recombinant human CD27L or portion thereof. In this context, a "recombinant protein" is a protein made using recombinant techniques using any techniques and methods known in the art, *i.e.*, through the expression of a recombinant nucleic acid as described herein. Methods and techniques for the production of recombinant proteins are well known in the art. Embodiments of the invention include recombinant antigen binding proteins that bind wild-type CD27L and variants thereof.

"Consisting essentially of" means that the amino acid sequence can vary by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15% relative to the recited SEQ ID NO: sequence and still retain biological activity, as described herein.

In some embodiments, the antigen binding proteins of the invention are isolated proteins or substantially pure proteins. An "isolated" protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, for example constituting at least about 5%, or at least about 50% by weight of the total protein in a given sample. It is understood that the isolated protein may constitute from 5 to 99.9% by weight of the total protein content depending on the circumstances. For example, the protein may be made at a significantly higher concentration through the use of an inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. The definition includes the production of an antigen binding protein in a wide variety of organisms and/or host cells that are known in the art.

For amino acid sequences, sequence identity and/or similarity is determined by using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith and Waterman, 1981, *Adv. Appl. Math.* 2:482, the sequence identity alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, *Proc. Nat. Acad. Sci. U.S.A.* 85:2444, computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.), the Best Fit sequence program described by Devereux *et al.*, 1984, *Nucl. Acid Res.* 12:387-395, preferably using the default settings, or by inspection. Preferably, percent identity is calculated by FastDB based upon the following parameters: mismatch penalty of 1; gap penalty of 1; gap size penalty of 0.33; and joining penalty of 30, "Current Methods in Sequence Comparison and Analysis," Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp 127-149 (1988), Alan R. Liss, Inc.

An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng &

Doolittle, 1987, *J. Mol. Evol.* 35:351-360; the method is similar to that described by Higgins and Sharp, 1989, *CABIOS* 5:151-153. Useful PILEUP parameters including a default gap weight of 3.00, a default gap length weight of 0.10, and weighted end gaps.

Another example of a useful algorithm is the BLAST algorithm, described in: Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-410; Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402; and Karin *et al.*, 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90:5873-5787. A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul *et al.*, 1996, *Methods in Enzymology* 266:460-480. WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T)=II. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

An additional useful algorithm is gapped BLAST as reported by Altschul *et al.*, 1993, *Nucl. Acids Res.* 25:3389-3402. Gapped BLAST uses BLOSUM-62 substitution scores; threshold T parameter set to 9; the two-hit method to trigger ungapped extensions, charges gap lengths of k a cost of 10+k; X_u set to 16, and X_g set to 40 for database search stage and to 67 for the output stage of the algorithms. Gapped alignments are triggered by a score corresponding to about 22 bits.

Generally, the amino acid homology, similarity, or identity between individual variant CDRs are at least 80% to the sequences depicted herein, and more typically with preferably increasing homologies or identities of at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and almost 100%. In a similar manner, "percent (%) nucleic acid sequence identity" with respect to the nucleic acid sequence of the binding proteins identified herein is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the coding sequence of the antigen binding protein. A specific method utilizes the BLASTN module of WU-BLAST-2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively.

Generally, the nucleic acid sequence homology, similarity, or identity between the nucleotide sequences encoding individual variant CDRs and the nucleotide sequences depicted herein are at least 80%, and more typically with preferably increasing homologies or identities of at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and almost 100%.

Thus, a "variant CDR" is one with the specified homology, similarity, or identity to the parent CDR of the invention, and shares biological function, including, but not limited to, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the specificity and/or activity of the parent CDR.

While the site or region for introducing an amino acid sequence variation is predetermined, the mutation *per se* need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed antigen binding protein CDR variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants is done using assays of antigen binding protein activities, such as CD27L binding.

Amino acid substitutions are typically of single residues; insertions usually will be on the order of from about one (1) to about twenty (20) amino acid residues, although considerably larger insertions may be tolerated. Deletions range from about one (1) to about twenty (20) amino acid residues, although in some cases deletions may be much larger.

Substitutions, deletions, insertions or any combination thereof may be used to arrive at a final derivative or variant. Generally these changes are done on a few amino acids to minimize the alteration of the molecule, particularly the immunogenicity and specificity of the antigen binding protein. However, larger changes may be tolerated in certain circumstances. Conservative substitutions are generally made in accordance with the following chart depicted as TABLE 1.

TABLE 1

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Ile
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those shown in TABLE 1. For example, substitutions may be made which more significantly affect: the structure of the polypeptide backbone in the area of the alteration, for example the alpha-helical or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the polypeptide's properties are those in which (a) a hydrophilic residue, *e.g.*, seryl or threonyl, is substituted for (or by) a hydrophobic residue, *e.g.*, leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, *e.g.*, lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, *e.g.*, glutamyl or aspartyl; or (d) a residue having a bulky side chain, *e.g.*, phenylalanine, is substituted for (or by) one not having a side chain, *e.g.*, glycine.

The variants typically exhibit the same qualitative biological activity and will elicit the same immune response as the naturally-occurring analogue, although variants

also are selected to modify the characteristics of the antigen binding protein proteins as needed. Alternatively, the variant may be designed such that the biological activity of the antigen binding protein is altered. For example, glycosylation sites may be altered or removed as discussed herein.

Other derivatives of CD27L antibodies within the scope of this invention include covalent or aggregative conjugates of CD27L antibodies, or fragments thereof, with other proteins or polypeptides, such as by expression of recombinant fusion proteins comprising heterologous polypeptides fused to the N-terminus or C-terminus of a CD27L antibody polypeptide. For example, the conjugated peptide may be a heterologous signal (or leader) polypeptide, *e.g.*, the yeast alpha-factor leader, or a peptide such as an epitope tag. CD27L antibody-containing fusion proteins can comprise peptides added to facilitate purification or identification of the CD27L antibody (*e.g.*, poly-His). A CD27L antibody polypeptide also can be linked to the FLAG peptide as described in Hopp *et al.*, *Bio/Technology* 6:1204, 1988, and U.S. Patent 5,011,912. The FLAG peptide is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody (mAb), enabling rapid assay and facile purification of expressed recombinant protein. Reagents useful for preparing fusion proteins in which the FLAG peptide is fused to a given polypeptide are commercially available (Sigma, St. Louis, MO).

Oligomers that contain one or more CD27L antibody polypeptides may be employed as CD27L antagonists. Oligomers may be in the form of covalently-linked or non-covalently-linked dimers, trimers, or higher oligomers. Oligomers comprising two or more CD27L antibody polypeptides are contemplated for use, with one example being a homodimer. Other oligomers include heterodimers, homotrimers, heterotrimers, homotetramers, heterotetramers, *etc.*

One embodiment is directed to oligomers comprising multiple CD27L antibody polypeptides joined *via* covalent or non-covalent interactions between peptide moieties fused to the CD27L antibody polypeptides. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of CD27L antibody polypeptides attached thereto, as described in more detail below.

In particular embodiments, the oligomers comprise from two to four CD27L antibody polypeptides. The CD27L antibody moieties of the oligomer may be in any of the forms described above, *e.g.*, variants or fragments. Preferably, the oligomers comprise CD27L antibody polypeptides that have CD27L binding activity.

In one embodiment, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, *e.g.*, by Ashkenazi *et al.*, 1991, PNAS USA 88:10535; Byrn *et al.*, 1990, Nature 344:677; and Hollenbaugh *et al.*, 1992 "Construction of Immunoglobulin Fusion Proteins", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1 - 10.19.11.

One embodiment of the present invention is directed to a dimer comprising two fusion proteins created by fusing a CD27L binding fragment of a CD27L antibody to the Fc region of an antibody. The dimer can be made by, for example, inserting a gene fusion encoding the fusion protein into an appropriate expression vector, expressing the gene fusion in host cells transformed with the recombinant expression vector, and allowing the expressed fusion protein to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield the dimer.

The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization also are included. Fusion proteins comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

One suitable Fc polypeptide, described in PCT application WO 93/10151 (hereby incorporated by reference), is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and in Baum *et al.*, 1994, *EMBO J.* 13:3992-4001. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino

acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors.

In other embodiments, the variable portion of the heavy and/or light chains of a CD27L antibody may be substituted for the variable portion of an antibody heavy and/or light chain.

Alternatively, the oligomer is a fusion protein comprising multiple CD27L antibody polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233.

Another method for preparing oligomeric CD27L antibody derivatives involves use of a leucine zipper. Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz *et al.*, 1988, *Science* 240:1759), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe *et al.*, 1994, *FEBS Letters* 344:191, hereby incorporated by reference. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow *et al.*, 1994, *Semin. Immunol.* 6:267-78. In one approach, recombinant fusion proteins comprising CD27L antibody fragment or derivative fused to a leucine zipper peptide are expressed in suitable host cells, and the soluble oligomeric CD27L antibody fragments or derivatives that form are recovered from the culture supernatant.

Covalent modifications of antigen binding proteins are included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antigen binding protein are introduced into the molecule by reacting specific amino acid residues of the antigen binding protein with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N=C=N--R'), where R and R' are optionally different alkyl

groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking antigen binding proteins to a water-insoluble support matrix or surface for use in a variety of methods. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, 1983, pp. 79-86), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the antigen binding protein included within the scope of this invention comprises altering the glycosylation pattern of the protein. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (*e.g.*, the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antigen binding protein is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antigen binding protein amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the antigen binding protein is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston, 1981, *CRC Crit. Rev. Biochem.*, pp. 259-306.

Removal of carbohydrate moieties present on the starting antigen binding protein may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking

sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin *et al.*, 1987, *Arch. Biochem. Biophys.* 259:52 and by Edge *et al.*, 1981, *Anal. Biochem.* 118:131. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, 1987, *Meth. Enzymol.* 138:350. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.*, 1982, *J. Biol. Chem.* 257:3105. Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of the antigen binding protein comprises linking the antigen binding protein to various nonproteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. In addition, as is known in the art, amino acid substitutions may be made in various positions within the antigen binding protein to facilitate the addition of polymers such as PEG.

In some embodiments, the covalent modification of the antigen binding proteins of the invention comprises the addition of one or more labels.

The term "labelling group" means any detectable label. Examples of suitable labelling groups include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent groups (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic groups (*e.g.*, horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent groups, biotinyl groups, or predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, the labelling group is coupled to the antigen binding protein *via* spacer arms of various lengths to reduce potential steric hindrance. Various methods for labelling proteins are known in the art and may be used in performing the present invention.

In general, labels fall into a variety of classes, depending on the assay in which they are to be detected: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic labels (*e.g.*, magnetic particles); c) redox active moieties; d) optical dyes;

enzymatic groups (*e.g.* horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase); e) biotinylated groups; and f) predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags, etc.). In some embodiments, the labelling group is coupled to the antigen binding protein *via* spacer arms of various lengths to reduce potential steric hindrance. Various methods for labelling proteins are known in the art and may be used in performing the present invention.

Specific labels include optical dyes, including, but not limited to, chromophores, phosphors and fluorophores, with the latter being specific in many instances. Fluorophores can be either "small molecule" fluores, or proteinaceous fluores.

By "fluorescent label" is meant any molecule that may be detected *via* its inherent fluorescent properties. Suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade BlueJ, Texas Red, IAEDANS, EDANS, BODIPY FL, LC Red 640, Cy 5, Cy 5.5, LC Red 705, Oregon green, the Alexa-Fluor dyes (Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660, Alexa Fluor 680), Cascade Blue, Cascade Yellow and R-phycoerythrin (PE) (Molecular Probes, Eugene, OR), FITC, Rhodamine, and Texas Red (Pierce, Rockford, IL), Cy5, Cy5.5, Cy7 (Amersham Life Science, Pittsburgh, PA). Suitable optical dyes, including fluorophores, are described in Molecular Probes Handbook by Richard P. Haugland, hereby expressly incorporated by reference.

Suitable proteinaceous fluorescent labels also include, but are not limited to, green fluorescent protein, including a Renilla, Ptilosarcus, or Aequorea species of GFP (Chalfie *et al.*, 1994, *Science* 263:802-805), EGFP (Clontech Laboratories, Inc., Genbank Accession Number U55762), blue fluorescent protein (BFP, Quantum Biotechnologies, Inc. 1801 de Maisonneuve Blvd. West, 8th Floor, Montreal, Quebec, Canada H3H 1J9; Stauber, 1998, *Biotechniques* 24:462-471; Heim *et al.*, 1996, *Curr. Biol.* 6:178-182), enhanced yellow fluorescent protein (EYFP, Clontech Laboratories, Inc.), luciferase (Ichiki *et al.*, 1993, *J. Immunol.* 150:5408-5417), β galactosidase (Nolan *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:2603-2607) and Renilla (WO92/15673, WO95/07463,

WO98/14605, WO98/26277, WO99/49019, U.S. Patent Nos. 5292658, 5418155, 5683888, 5741668, 5777079, 5804387, 5874304, 5876995, 5925558). All of the above-cited references are expressly incorporated herein by reference.

Polynucleotides Encoding CD27L Antigen Binding Proteins

Encompassed within the invention are nucleic acids encoding CD27L antigen binding proteins, including antibodies, as defined herein. Preferred nucleic acids include those that encode the exemplary light and heavy chains described herein.

An exemplary nucleic acid encoding Ab1 LC is a nucleic acid comprising the sequence set forth in SEQ ID NO:49.

An exemplary nucleic acid encoding Ab2 LC is a nucleic acid comprising the sequence set forth in SEQ ID NO:50.

An exemplary nucleic acid encoding Ab4 LC is a nucleic acid comprising the sequence set forth in SEQ ID NO:51.

An exemplary nucleic acid encoding Ab5 LC is a nucleic acid comprising the sequence set forth in SEQ ID NO:52.

An exemplary nucleic acid encoding Ab6 LC is a nucleic acid comprising the sequence set forth in SEQ ID NO:53.

An exemplary nucleic acid encoding Ab7 LC is a nucleic acid comprising the sequence set forth in SEQ ID NO:54.

An exemplary nucleic acid encoding Ab8 LC is a nucleic acid comprising the sequence set forth in SEQ ID NO:55.

An exemplary nucleic acid encoding Ab1 HC is a nucleic acid comprising the sequence set forth in SEQ ID NO:3.

An exemplary nucleic acid encoding Ab2 HC is a nucleic acid comprising the sequence set forth in SEQ ID NO:4.

An exemplary nucleic acid encoding Ab4 HC is a nucleic acid comprising the sequence set forth in SEQ ID NO:5.

An exemplary nucleic acid encoding Ab5 HC is a nucleic acid comprising the sequence set forth in SEQ ID NO:6.

An exemplary nucleic acid encoding Ab6 HC is a nucleic acid comprising the sequence set forth in SEQ ID NO:7.

An exemplary nucleic acid encoding Ab7 HC is a nucleic acid comprising the sequence set forth in SEQ ID NO:8.

An exemplary nucleic acid encoding Ab8 HC is a nucleic acid comprising the sequence set forth in SEQ ID NO:9.

Aspects of the invention include polynucleotide variants (*e.g.*, due to degeneracy) that encode the amino acid sequences described herein.

Aspects of the invention include a variety of embodiments including, but not limited to, the following exemplary embodiments.

An isolated polynucleotide, wherein said polynucleotide encodes one or more polypeptides comprising an amino acid sequence selected from the group consisting of:

A. 1. a light chain variable domain sequence that is at least 90% identical to a light chain variable domain sequence set forth in SEQ ID NOs:63-70;

2. a heavy chain variable domain sequence that is at least 90% identical to a heavy chain variable domain sequence set forth in SEQ ID NOs:17-24;

3. a light chain variable domain of (1) and a heavy chain variable domain of (2); and

B. a light chain variable domain comprising a CDR1, CDR2, CDR3 and/or a heavy chain variable domain comprising a CDR1, CDR2, CDR3 that differ by no more than a total of three amino acid additions, substitutions, and/or deletions in each CDR from the following sequences:

1. a light chain CDR1 (SEQ ID NO:71), CDR2 (SEQ ID NO:79), CDR3 (SEQ ID NO:87) or a heavy chain CDR1 (SEQ ID NO:25), CDR2 (SEQ ID NO:33), CDR3 (SEQ ID NO:41) of Ab1;

2. a light chain CDR1 (SEQ ID NO:72), CDR2 (SEQ ID NO:80), CDR3 (SEQ ID NO:88) or a heavy chain CDR1 (SEQ ID NO:26), CDR2 (SEQ ID NO:34), CDR3 (SEQ ID NO:42) of Ab2;

3. a light chain CDR1 (SEQ ID NO:73), CDR2 (SEQ ID NO:81), CDR3 (SEQ ID NO:89) or a heavy chain CDR1 (SEQ ID NO:27), CDR2 (SEQ ID NO:35), CDR3 (SEQ ID NO:43) of Ab3;

4. a light chain CDR1 (SEQ ID NO:74), CDR2 (SEQ ID NO:82), CDR3 (SEQ ID NO:90) or a heavy chain CDR1 (SEQ ID NO:28), CDR2 (SEQ ID NO:36), CDR3 (SEQ ID NO:44) of Ab4;

5. a light chain CDR1 (SEQ ID NO:75), CDR2 (SEQ ID NO:83), CDR3 (SEQ ID NO:91) or a heavy chain CDR1 (SEQ ID NO:29), CDR2 (SEQ ID NO:37), CDR3 (SEQ ID NO:45) of Ab5;

6. a light chain CDR1 (SEQ ID NO:76), CDR2 (SEQ ID NO:84), CDR3 (SEQ ID NO:92) or a heavy chain CDR1 (SEQ ID NO:30), CDR2 (SEQ ID NO:38), CDR3 (SEQ ID NO:46) of Ab6;

7. a light chain CDR1 (SEQ ID NO:77), CDR2 (SEQ ID NO:85), CDR3 (SEQ ID NO:93) or a heavy chain CDR1 (SEQ ID NO:31), CDR2 (SEQ ID NO:39), CDR3 (SEQ ID NO:47) of Ab7; and

8. a light chain CDR1 (SEQ ID NO:78), CDR2 (SEQ ID NO:86), CDR3 (SEQ ID NO:94) or a heavy chain CDR1 (SEQ ID NO:32), CDR2 (SEQ ID NO:40), CDR3 (SEQ ID NO:48) of Ab8.

In preferred embodiments, the polypeptide encoded by the isolated nucleic acid is a component of an antigen binding protein that binds CD27L.

Nucleotide sequences corresponding to the amino acid sequences described herein, to be used as probes or primers for the isolation of nucleic acids or as query sequences for database searches, can be obtained by "back-translation" from the amino acid sequences, or by identification of regions of amino acid identity with polypeptides for which the coding DNA sequence has been identified. The well-known polymerase chain reaction (PCR) procedure can be employed to isolate and amplify a DNA sequence encoding a CD27L antigen binding proteins or a desired combination of CD27L antigen binding protein polypeptide fragments. Oligonucleotides that define the desired termini of the combination of DNA fragments are employed as 5' and 3' primers. The oligonucleotides can additionally contain recognition sites for restriction endonucleases, to facilitate insertion of the amplified combination of DNA fragments into an expression vector. PCR techniques are described in Saiki et al., *Science* 239:487 (1988); *Recombinant DNA Methodology*, Wu et al., eds., Academic Press, Inc., San Diego

(1989), pp. 189-196; and *PCR Protocols: A Guide to Methods and Applications*, Innis *et. al.*, eds., Academic Press, Inc. (1990).

Nucleic acid molecules of the invention include DNA and RNA in both single-stranded and double-stranded form, as well as the corresponding complementary sequences. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. The nucleic acid molecules of the invention include full-length genes or cDNA molecules as well as a combination of fragments thereof. The nucleic acids of the invention are preferentially derived from human sources, but the invention includes those derived from non-human species, as well.

An "isolated nucleic acid" is a nucleic acid that has been separated from adjacent genetic sequences present in the genome of the organism from which the nucleic acid was isolated, in the case of nucleic acids isolated from naturally-occurring sources. In the case of nucleic acids synthesized enzymatically from a template or chemically, such as PCR products, cDNA molecules, or oligonucleotides for example, it is understood that the nucleic acids resulting from such processes are isolated nucleic acids. An isolated nucleic acid molecule refers to a nucleic acid molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. In one preferred embodiment, the nucleic acids are substantially free from contaminating endogenous material. The nucleic acid molecule has preferably been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods (such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region.

The present invention also includes nucleic acids that hybridize under moderately stringent conditions, and more preferably highly stringent conditions, to nucleic acids

encoding CD27L antigen binding proteins as described herein. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by Sambrook,, Fritsch, and Maniatis (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and Current Protocols in Molecular Biology, 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA. One way of achieving moderately stringent conditions involves the use of a prewashing solution containing 5 x SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6 x SSC, and a hybridization temperature of about 55 degrees C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of about 42 degrees C), and washing conditions of about 60 degrees C, in 0.5 x SSC, 0.1% SDS. Generally, highly stringent conditions are defined as hybridization conditions as above, but with washing at approximately 68 degrees C, 0.2 x SSC, 0.1% SDS. SSPE (1xSSPE is 0.15M NaCl, 10 mM NaH.sub.2 PO.sub.4, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete. It should be understood that the wash temperature and wash salt concentration can be adjusted as necessary to achieve a desired degree of stringency by applying the basic principles that govern hybridization reactions and duplex stability, as known to those skilled in the art and described further below (see, e.g., Sambrook et al., 1989). When hybridizing a nucleic acid to a target nucleic acid of unknown sequence, the hybrid length is assumed to be that of the hybridizing nucleic acid. When nucleic acids of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the nucleic acids and identifying the region or regions of optimal sequence complementarity. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5 to 10.degrees C less than the melting temperature (Tm) of the hybrid, where Tm is determined according to the following equations. For hybrids less than 18 base pairs in length, Tm (degrees C) = 2(# of A + T bases) + 4(# of #G + C bases). For hybrids above 18 base pairs in length, Tm (degrees C) = 81.5 + 16.6($\log_{10}$ [Na^+]) + 0.41(% G +

C) - (600/N), where N is the number of bases in the hybrid, and [Na^+] is the concentration of sodium ions in the hybridization buffer ([Na^+] for 1xSSC = 0.165M). Preferably, each such hybridizing nucleic acid has a length that is at least 15 nucleotides (or more preferably at least 18 nucleotides, or at least 20 nucleotides, or at least 25 nucleotides, or at least 30 nucleotides, or at least 40 nucleotides, or most preferably at least 50 nucleotides), or at least 25% (more preferably at least 50%, or at least 60%, or at least 70%, and most preferably at least 80%) of the length of the nucleic acid of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, and most preferably at least 99.5%) with the nucleic acid of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing nucleic acids when aligned so as to maximize overlap and identity while minimizing sequence gaps as described in more detail above.

The variants according to the invention are ordinarily prepared by site specific mutagenesis of nucleotides in the DNA encoding the antigen binding protein, using cassette or PCR mutagenesis or other techniques well known in the art, to produce DNA encoding the variant, and thereafter expressing the recombinant DNA in cell culture as outlined herein. However, antigen binding protein fragments comprising variant CDRs having up to about 100-150 residues may be prepared by *in vitro* synthesis using established techniques. The variants typically exhibit the same qualitative biological activity as the naturally occurring analogue, *e.g.*, binding to CD27L, although variants can also be selected which have modified characteristics as will be more fully outlined below.

As will be appreciated by those in the art, due to the degeneracy of the genetic code, an extremely large number of nucleic acids may be made, all of which encode the CDRs (and heavy and light chains or other components of the antigen binding protein) of the present invention. Thus, having identified a particular amino acid sequence, those skilled in the art could make any number of different nucleic acids, by simply modifying

the sequence of one or more codons in a way which does not change the amino acid sequence of the encoded protein.

The present invention also provides expression systems and constructs in the form of plasmids, expression vectors, transcription or expression cassettes which comprise at least one polynucleotide as above. In addition, the invention provides host cells comprising such expression systems or constructs.

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

Optionally, the vector may contain a "tag"-encoding sequence, *i.e.*, an oligonucleotide molecule located at the 5' or 3' end of the CD27L antigen binding protein coding sequence; the oligonucleotide sequence encodes polyHis (such as hexaHis), or another "tag" such as FLAG, HA (hemaglutinin influenza virus), or *myc*, for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification or detection of the CD27L antigen binding protein from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified CD27L antigen binding protein by various means such as using certain peptidases for cleavage.

Flanking sequences may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (*i.e.*, from a species other than the host cell species or strain), hybrid (*i.e.*, a combination of flanking sequences from more than one source), synthetic or native. As such, the source of a flanking sequence may be any prokaryotic or

eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence is functional in, and can be activated by, the host cell machinery.

Flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein will have been previously identified by mapping and/or by restriction endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of a flanking sequence may be known. Here, the flanking sequence may be synthesized using the methods described herein for nucleic acid synthesis or cloning.

Whether all or only a portion of the flanking sequence is known, it may be obtained using polymerase chain reaction (PCR) and/or by screening a genomic library with a suitable probe such as an oligonucleotide and/or flanking sequence fragment from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, Qiagen® column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (New England Biolabs, Beverly, MA) is suitable for most gram-negative bacteria, and various viral origins (*e.g.*, SV40, polyoma, adenovirus, vesicular stomatitus virus (VSV), or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it also contains the virus early promoter).

A transcription termination sequence is typically located 3' to the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly-T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, tetracycline, or kanamycin for prokaryotic host cells; (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex or defined media. Specific selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. Advantageously, a neomycin resistance gene may also be used for selection in both prokaryotic and eukaryotic host cells.

Other selectable genes may be used to amplify the gene that will be expressed. Amplification is the process wherein genes that are required for production of a protein critical for growth or cell survival are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and promoterless thymidine kinase genes. Mammalian cell transformants are placed under selection pressure wherein only the transformants are uniquely adapted to survive by virtue of the selectable gene present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively increased, thereby leading to the amplification of both the selectable gene and the DNA that encodes another gene, such as an antigen binding protein antibody that binds to CD27L polypeptide. As a result, increased quantities of a polypeptide such as an CD27L antigen binding protein are synthesized from the amplified DNA.

A ribosome-binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding

sequence of the polypeptide to be expressed. In certain embodiments, one or more coding regions may be operably linked to an internal ribosome binding site (IRES), allowing translation of two open reading frames from a single RNA transcript.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various pre- or prosequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add prosequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the amino-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired polypeptide, if the enzyme cuts at such area within the mature polypeptide.

Expression and cloning vectors of the invention will typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding the CD27L antigen binding protein. Promoters are untranscribed sequences located upstream (*i.e.*, 5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control transcription of the structural gene. Promoters are conventionally grouped into one of two classes: inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, uniformly transcribe gene to which they are operably linked, that is, with little or no control over gene expression. A large number of promoters, recognized by a variety of potential host cells, are well known. A suitable promoter is operably linked to the DNA encoding heavy chain or light chain comprising an CD27L antigen binding protein of the invention by removing the promoter from the source DNA by restriction enzyme digestion and inserting the desired promoter sequence into the vector.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use

with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, retroviruses, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, for example, heat-shock promoters and the actin promoter.

Additional promoters which may be of interest include, but are not limited to: SV40 early promoter (Benoist and Chambon, 1981, *Nature* 290:304-310); CMV promoter (Thornsen *et al.*, 1984, *Proc. Natl. Acad. U.S.A.* 81:659-663); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, *Cell* 22:787-797); herpes thymidine kinase promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1444-1445); promoter and regulatory sequences from the metallothionine gene Prinster *et al.*, 1982, *Nature* 296:39-42); and prokaryotic promoters such as the beta-lactamase promoter (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. U.S.A.* 75:3727-3731); or the tac promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:21-25). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region that is active in pancreatic acinar cells (Swift *et al.*, 1984, *Cell* 38:639-646; Ornitz *et al.*, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); the insulin gene control region that is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122); the immunoglobulin gene control region that is active in lymphoid cells (Grosschedl *et al.*, 1984, *Cell* 38:647-658; Adames *et al.*, 1985, *Nature* 318:533-538; Alexander *et al.*, 1987, *Mol. Cell. Biol.* 7:1436-1444); the mouse mammary tumor virus control region that is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, *Cell* 45:485-495); the albumin gene control region that is active in liver (Pinkert *et al.*, 1987, *Genes and Devel.* 1 :268-276); the alpha-feto-protein gene control region that is active in liver (Krumlauf *et al.*, 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer *et al.*, 1987, *Science* 253:53-58); the alpha 1-antitrypsin gene control region that is active in liver (Kelsey *et al.*, 1987, *Genes and Devel.* 1:161-171); the beta-globin gene control region that is active in myeloid cells (Mogram *et al.*, 1985, *Nature* 315:338-340; Kollias *et al.*, 1986, *Cell* 46:89-94); the

myelin basic protein gene control region that is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, *Cell* 48:703-712); the myosin light chain-2 gene control region that is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286); and the gonadotropic releasing hormone gene control region that is active in the hypothalamus (Mason *et al.*, 1986, *Science* 234:1372-1378).

An enhancer sequence may be inserted into the vector to increase transcription of DNA encoding light chain or heavy chain comprising a CD27L antigen binding protein of the invention by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent, having been found at positions both 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (*e.g.*, globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus is used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers known in the art are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be positioned in the vector either 5' or 3' to a coding sequence, it is typically located at a site 5' from the promoter. A sequence encoding an appropriate native or heterologous signal sequence (leader sequence or signal peptide) can be incorporated into an expression vector, to promote extracellular secretion of the antibody. The choice of signal peptide or leader depends on the type of host cells in which the antibody is to be produced, and a heterologous signal sequence can replace the native signal sequence. Examples of signal peptides that are functional in mammalian host cells include the following: the signal sequence for interleukin-7 (IL-7) described in US Patent No. 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman *et al.*,1984, *Nature* 312:768; the interleukin-4 receptor signal peptide described in EP Patent No. 0367 566; the type I interleukin-1 receptor signal peptide described in U.S. Patent No. 4,968,607; the type II interleukin-1 receptor signal peptide described in EP Patent No. 0 460 846.

The vector may contain one or more elements that facilitate expression when the vector is integrated into the host cell genome. Examples include an EASE element (Aldrich et al. 2003 *Biotechnol Prog.* 19:1433-38) and a matrix attachment region

(MAR). MARs mediate structural organization of the chromatin and may insulate the integrated vactor from "position" effect. Thus, MARs are particularly useful when the vector is used to create stable transfectants. A number of natural and synthetic MAR-containing nucleic acids are known in the art, *e.g.*, U.S. Pat. Nos. 6,239,328; 7,326,567; 6,177,612; 6,388,066; 6,245,974; 7,259,010; 6,037,525; 7,422,874; 7,129,062.

Expression vectors of the invention may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the flanking sequences described herein are not already present in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

After the vector has been constructed and a nucleic acid molecule encoding light chain, a heavy chain, or a light chain and a heavy chain comprising an CD27L antigen binding sequence has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression. The transformation of an expression vector for an CD27L antigen binding protein into a selected host cell may be accomplished by well known methods including transfection, infection, calcium phosphate co-precipitation, electroporation, microinjection, lipofection, DEAE-dextran mediated transfection, or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, 2001, *supra*.

A host cell, when cultured under appropriate conditions, synthesizes an CD27L antigen binding protein that can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). The selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity (such as glycosylation or phosphorylation) and ease of folding into a biologically active molecule. A host cell may be eukaryotic or prokaryotic.

Mammalian cell lines available as hosts for expression are well known in the art and include, but are not limited to, immortalized cell lines available from the American

Type Culture Collection (ATCC) and any cell lines used in an expression system known in the art can be used to make the recombinant polypeptides of the invention. In general, host cells are transformed with a recombinant expression vector that comprises DNA encoding a desired anti- CD27L antibody polypeptide. Among the host cells that may be employed are prokaryotes, yeast or higher eukaryotic cells. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or bacilli. Higher eukaryotic cells include insect cells and established cell lines of mammalian origin. Examples of suitable mammalian host cell lines include the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman *et al.*, 1981, Cell 23:175), L cells, 293 cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells, or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (Rasmussen *et al.*, 1998, *Cytotechnology* 28: 31), HeLa cells, BHK (ATCC CRL 10) cell lines, and the CVI/EBNA cell line derived from the African green monkey kidney cell line CVI (ATCC CCL 70) as described by McMahan *et al.*, 1991, EMBO J. 10: 2821, human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Optionally, mammalian cell lines such as HepG2/3B, KB, NIH 3T3 or S49, for example, can be used for expression of the polypeptide when it is desirable to use the polypeptide in various signal transduction or reporter assays. Alternatively, it is possible to produce the polypeptide in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeasts include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous polypeptides. Suitable bacterial strains include Escherichia coli, Bacillus subtilis, Salmonella typhimurium, or any bacterial strain capable of expressing heterologous polypeptides. If the polypeptide is made in yeast or bacteria, it may be desirable to modify the polypeptide produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional polypeptide. Such covalent attachments can be accomplished using known chemical or enzymatic methods. The polypeptide can also be produced by operably linking the isolated nucleic acid of the invention to suitable control sequences in one or more insect

expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, Calif., U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), and Luckow and Summers, *Bio/Technology* 6:47 (1988). Cell-free translation systems could also be employed to produce polypeptides using RNAs derived from nucleic acid constructs disclosed herein. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels *et al.* (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985). A host cell that comprises an isolated nucleic acid of the invention, preferably operably linked to at least one expression control sequence, is a "recombinant host cell".

In certain embodiments, cell lines may be selected through determining which cell lines have high expression levels and constitutively produce antigen binding proteins with CD27L binding properties. In another embodiment, a cell line from the B cell lineage that does not make its own antibody but has a capacity to make and secrete a heterologous antibody can be selected.

Antibody Drug Conjugates

Embodiments of the invention include antibody drug conjugates (ADCs). Generally the ADC comprises an antibody conjugated to a chemotherapeutic agent, e.g., a cytotoxic agent, a cytostatic agent, a toxin, or a radioactive agent. A linker molecule can be used to conjugate the drug to the antibody. A wide variety of linkers and drugs useful in ADC technology are known in the art and may be used in embodiments of the present invention. (See US20090028856; US2009/0274713; US2007/0031402; WO2005/084390; WO2009/099728; US5208020; US5416064; US5475092; 5585499; 6436931; 6372738; and 6340701, all incorporated herein by reference).

The antibody drug conjugates may be prepared by in vitro methods. In order to link a drug or prodrug to the antibody, a linking group is used. Suitable linking groups are well known in the art and include disulfide groups, acid labile groups, photolabile

groups, peptidase labile groups, and esterase labile groups. Preferred linking groups are disulfide groups. For example, conjugates can be constructed using a disulfide exchange reaction between the antibody and the drug or prodrug. The drug molecules also can be linked to a cell binding agent through an intermediary carrier molecule such as serum albumin.

In certain embodiments, the cell binding agent is modified by reacting a bifunctional crosslinking reagent with the cell binding agent, thereby resulting in the covalent attachment of a linker molecule to the cell binding agent. As used herein, a "bifunctional crosslinking reagent" or "linker" is any chemical moiety that covalently links a cell binding agent to a drug, such as the drugs described herein. In a particular embodiment of the invention, a portion of the linking moiety is provided by the drug. In this respect, the drug comprises a linking moiety that is part of a larger linker molecule that is used to join the cell binding agent to the drug. For example, to form the maytansinoid DM1, the side chain at the C-3 hydroxyl group of maytansine is modified to have a free sulfhydryl group (SH). This thiolated form of maytansine can react with a modified cell-binding agent to form a conjugate. Therefore, the final linker is assembled from two components, one of which is provided by the crosslinking reagent, while the other is provided by the side chain from DM1.

Any suitable bifunctional crosslinking reagent can be used in connection with the invention, so long as the linker reagent provides for retention of the therapeutic, e.g., cytotoxicity, and targeting characteristics of the drug and the cell binding agent, respectively. Preferably, the linker molecule joins the drug to the cell binding agent through chemical bonds (as described above), such that the drug and the cell binding agent are chemically coupled (e.g., covalently bonded) to each other. Linkers

In certain embodiments, the ADC comprises a linker made up of one or more linker components. Preferably the drug is linked to a cell binding agent through a disulfide bond. The linker molecule comprises a reactive chemical group that can react with the cell binding agent. Preferred reactive chemical groups for reaction with the cell binding agent are N-succinimidyl esters and N-sulfosuccinimidyl esters. Additionally the linker molecule comprises a reactive chemical group, preferably a dithiopyridyl group, that can react with the drug to form a disulfide bond. Exemplary linker molecules

include, for example, N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) (see, e.g., Carlsson et al., Biochem. J., 173, 723-737 (1978)), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB) (see, e.g., U.S. Pat. No. 4,563,304) and N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP) (see, e.g., CAS Registry number 341498-08-6).Additional exemplary linker components include 6-maleimidocaproyl, maleimidopropanoyl, valine-citrulline, alanine-phenylalanine, p-aminobenzyloxycarbonyl, and those resulting from conjugation with linker reagents, including, but not limited to, N-succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("SMCC," also referred to herein also as "MCC"), and N-succinimidyl (4-iodo-acetyl) aminobenzoate ("SIAB").

Linkers may be a "cleavable" linker or a "non-cleavable" linker (Ducry and Stump, *Bioconjugate Chem.* 2010, *21*, 5-13; incorporated herein by reference in its entirety) Cleavable linkers are designed to release the drug when subjected to certain environment factors, e.g., when internalized into the target cell. Cleavable linkers include acid labile linkers, protease sensitive linkers, photolabile linkers, dimethyl linker or disulfide-containing linkers. Non-cleavable linkers tend to remain covalently associated with at least one amino acid of the antibody and the drug upon internalization by and degradation within the target cell. A non-cleavable linker is any chemical moiety that is capable of linking a drug, such as a maytansinoid (e.g., DM1, and the like), a taxane, or a CC-1065 analog, to a cell binding agent in a stable, covalent manner. Thus, non-cleavable linkers are substantially resistant to acid-induced cleavage, light-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage, at conditions under which the drug or the cell binding agent remains active.

Suitable crosslinking reagents that form non-cleavable linkers between a drug and the cell-binding agent are well known in the art. Examples of non-cleavable linkers include linkers having an N-succinimidyl ester or N-sulfosuccinimidyl ester moiety for reaction with the cell binding agent, as well as a maleimido- or haloacetyl-based moiety for reaction with the drug. Crosslinking linker reagents comprising a maleimido-based moiety include N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproa- te), which is a "long chain" analog of SMCC (LC--SMCC), x-maleimidoundecanoic acid N-

succinimidyl ester (KMUA), .gamma.-maleimidobutyric acid N-succinimidyl ester (GMBS), .epsilon.-maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(.alpha.-maleimidoacetoxy)-succinimide ester (AMAS), succinimidyl-6-(.beta.-maleimidopropionamido)hexanoate (SMPH), N-succinimidyl 4-(p-maleimidophenyl)-butyrate (SMPB), and N-(p-maleimidophenyl)isocyanate (PMPI). Cross-linking reagents comprising a haloacetyl-based moiety include N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB), N-succinimidyl iodoacetate (SIA), N-succinimidyl bromoacetate (SBA), and N-succinimidyl 3-(bromoacetamido)propionate (SBAP). An exemplary preferred non-cleavable linker is MCC.

Other crosslinking reagent linkers lacking a sulfur atom that form non-cleavable linkers can also be used in the inventive method. Such linkers can be derived from dicarboxylic acid based moieties. Suitable dicarboxylic acid based moieties include, but are not limited to, alpha,omega-dicarboxylic acids of the general formula (IX):

$$HOOC\text{--}X_l\text{--}Y_n\text{--}Z_m\text{--}COOH \qquad (IX),$$

wherein X is a linear or branched alkyl, alkenyl, or alkynyl group having 2 to 20 carbon atoms, Y is a cycloalkyl or cycloalkenyl group bearing 3 to 10 carbon atoms, Z is a substituted or unsubstituted aromatic group bearing 6 to 10 carbon atoms, or a substituted or unsubstituted heterocyclic group wherein the hetero atom is selected from N, O or S, and wherein l, m, and n are each 0 or 1, provided that l, m, and n are all not zero at the same time.

Many of the non-cleavable linkers disclosed herein are described in detail in U.S. Patent Application Publication 2005/0169933 A1.

Examples of suitable cleavable linkers include disulfide linkers, acid labile linkers, photolabile linkers, peptidase labile linkers, and esterase labile linkers. Disulfide containing linkers are linkers cleavable through disulfide exchange, which can occur under physiological conditions. Acid labile linkers are linkers cleavable at acid pH. For example, certain intracellular compartments, such as endosomes and lysosomes, have an acidic pH (pH 4-5), and provide conditions suitable to cleave acid labile linkers. Photo

labile linkers are useful at the body surface and in many body cavities that are accessible to light. Furthermore, infrared light can penetrate tissue. Peptidase labile linkers can be used to cleave certain peptides inside or outside cells (see e.g., Trouet et al., Proc. Natl. Acad. Sci. USA, 79, 626-629 (1982), and Umemoto et al., Int. J. Cancer, 43, 677-684 (1989)). Drugs

In certain embodiments, the antibody is conjugated to a chemotherapeutic agent. Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclophosphamide (CYTOXAN.TM.); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics, such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin .gamma1 and calicheamicin theta I, see, e.g., Angew Chem. Intl. Ed. Engl. 33:183-186 (1994); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin; chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, nitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin,

zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens, such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals, such as aminoglutethimide, mitotane, trilostane; folic acid replenisher, such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK.RTM.; razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL.TM., Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE.RTM., Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors, such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens, such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; siRNA and pharmaceutically acceptable salts, acids or derivatives of any of the above. Other chemotherapeutic agents

that can be used with the present invention are disclosed in US Publication No. 20080171040 or US Publication No. 20080305044 and are incorporated in their entirety by reference.

It is contemplated that an antibody may be conjugated to two or more different chemotherapeutic agents or a pharmaceutical composition may comprise a mixture of antibodies wherein the antibody component is identical except for being conjugated to a different chemotherapeutic agent. Such embodiments may be useful for targeting multiple biological pathways with a target cell.

In preferred embodiments, the ADC comprises an antibody conjugated to one or more maytansinoid molecules, which are mitotic inhibitors that act by inhibiting tubulin polymerization. Maytansinoids, including various modifications, are described in US Pat. Nos. 3896111; 4151042; 4137230; 4248870; 4256746; 4260608; 4265814; 4294757; 4307016; 4308268; 4309428; 4313946; 4315929; 4317821; 4322348; 4331598; 4361650; 4364866; 4424219; 4450254; 4362663; 4371533; and WO 2009/099728. Maytansinoid drug moieties may be isolated from natural sources, produced using recombinant technology, or prepared synthetically. Exemplary maytansinoids include C-19-dechloro (US Pat No. 4256746), C-20-hydroxy (or C-20-demethyl) +/- C-19-dechloro (US Pat. Nos. 4307016 and 4361650), C-20-demethoxy (or C-20-acyloxy (-OCOR), +/-dechrolo (US Pat. No. 4294757), C-9-SH (US Pat. No. 4,424,219), C-14-alkoxymethyl (demethoxy/CH2OR) (U.S. Pat. No. 4,331,598), C-14-hydroxymethyl or acyloxymethyl (CH2OH or CH2OAc) (U.S. Pat. No. 4,450,254), C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866), C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929), C-18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348), and 4,5-deoxy (U.S. Pat. No. 4,371,533).

Various positions on maytansinoid compounds may be used as the linkage position, depending upon the type of link desired. For example, for forming an ester linkage, the C-3 position having a hydroxyl group, the C-14 position modified with hydrozymethyl, the C-15 position modified with a hydroxyl a group, and the C-20 position having a hydroxyl group are all suitable (US Pat. Nos. 5208020, RE39151, and 6913748; US Patent Appl. Pub. Nos. 20060167245 and 20070037972, and WO 2009099728).

Preferred maytansinoids include those known in the art as DM1, DM3, and DM4 (US Pat. Appl. Pub. Nos. 2009030924 and 20050276812, incorporated herein by reference).

ADCs containing maytansinoids, methods of making such ADCs, and their therapeutic use are disclosed in US Patent Nos. 5208020 and 5416064, US Pat. Appl. Pub. No. 20050276812, and WO 2009099728 (all incorporated by reference herein). Linkers that are useful for making maytansinoid ADCs are know in the art (US Pat. No. 5208020 and US Pat. Appl. Pub. Nos. 2005016993 and 20090274713; all incorporated herein by reference). Maytansinoid ADCs comprising an SMCC linker may be prepared as disclosed in US Pat. Publ. No. 2005/0276812.

In certain embodiments, the ADC comprises an antibody conjugated to DM1 with an SMCC linker. Preferred embodiments include Ab1-SMCC-DM1, Ab2-SMCC-DM1, Ab3-SMCC-DM1, Ab4-SMCC-DM1, Ab5-SMCC-DM1, Ab6-SMCC-DM1, Ab7-SMCC-DM1, and Ab8-SMCC-DM1.

Drug Loading

An ADC may have 1 to 20 chemotherapeutic agents per antibody. Compositions of ADCs may be characterized by the average number of drug moieties per antibody molecule in the composition. The average number of drug moieties may be determined by conventional means such as mass spectrometry, immunoassay, and HPLC. In some instances, a homogeneous ADC population may be separated and purified by means of reverse phase HPLC or electrophoresis. Thus, pharmaceutical ADC compositions may contain a heterogeneous or homogeneous population of antibodies linked to 1, 2, 3, 4, 5, 6, 7 or more drug moieties.

In preferred embodiments, the ADC comprises as antibody conjugated to one or more DM1 molecules. Embodiments of the invention include compositions comprising an average of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20 DM1 molecules per antibody. Preferred ADC compositions are those comprising an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, or Ab8 having on average between 1 and 10 DM1 molecules per antibody, those comprising antibodies having on average between 3 and 7 DM1 molecules per antibody, and those comprising antibodies

having on average between 4 and 6 DM1 molecules, including an average of about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9 and about 6.0 DM1 molecules per antibody.

Effector Function-Enhanced Antibodies

One of the functions of the Fc portion of an antibody is to communicate to the immune system when the antibody binds its target. This is considered "effector function." Communication leads to antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and/or complement dependent cytotoxicity (CDC). ADCC and ADCP are mediated through the binding of the Fc to Fc receptors on the surface of cells of the immune system. CDC is mediated through the binding of the Fc with proteins of the complement system, e.g., C1q.

The IgG subclasses vary in their ability to mediate effector functions. For example IgG1 is much superior to IgG2 and IgG4 at mediating ADCC and CDC. Thus, in embodiments wherein a cell expressing CD27L is targeted for destruction, an anti-CD27L IgG1 antibody would be preferred.

The effector function of an antibody can be increased, or decreased, by introducing one or more mutations into the Fc. Embodiments of the invention include antigen binding proteins, e.g., antibodies, having an Fc engineered to increase effector function (U.S. 7,317,091 and Strohl, *Curr. Opin. Biotech.*, 20:685-691, 2009; both incorporated herein by reference in its entirety). . Exemplary IgG1 Fc molecules having increased effector function include (based on the Kabat numbering scheme) those have the following substitutions: S239D/I332E

S239D/A330S/I332E

S239D/A330L/I332E

S298A/D333A/K334A

P247I/A339D

P247I/A339Q

D280H/K290S

D280H/K290S/S298D

D280H/K290S/S298V
F243L/R292P/Y300L
F243L/R292P/Y300L/P396L
F243L/R292P/Y300L/V305I/P396L
G236A/S239D/I332E
K326A/E333A
K326W/E333S
K290E/S298G/T299A
K290N/S298G/T299A
K290E/S298G/T299A/K326E
K290N/S298G/T299A/K326E

Further embodiments of the invention include antigen binding proteins, e.g., antibodies, having an Fc engineered to decrease effector function. Exemplary Fc molecules having decreased effector function include (based on the Kabat numbering scheme) those have the following substitutions:
N297A (IgG1)
L234A/L235A (IgG1)
V234A/G237A (IgG2)
L235A/G237A/E318A (IgG4)
H268Q/V309L/A330S/A331S (IgG2)
C220S/C226S/C229S/P238S (IgG1)
C226S/C229S/E233P/L234V/L235A (IgG1)
L234F/L235E/P331S (IgG1)
S267E/L328F (IgG1)

Another method of increasing effector function of IgG Fc-containing proteins is by reducing the fucosylation of the Fc. Removal of the core fucose from the biantennary complex-type oligosachharides attached to the Fc greatly increased ADCC effector function without altering antigen binding or CDC effector function. Several ways are known for reducing or abolishing fucosylation of Fc-containing molecules, e.g.,

antibodies. These include recombinant expression in certain mammalian cell lines including a FUT8 knockout cell line, variant CHO line Lec13, rat hybridoma cell line YB2/0, a cell line comprising a small interfering RNA specifically against the FUT8 gene, and a cell line coexpressing B-1,4-*N*-acetylglucosaminyltransferase III and Golgi α-mannosidase II. Alternatively, the Fc-containing molecule may be expressed in a non-mammalian cell such as a plant cell, yeast, or prokaryotic cell, e.g., E. coli. Thus, in certain embodiments of the invention, a composition comprises an antibody, e.g., Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, or Ab8, having reduced fucosylation or lacking fucosylation altogether.

Pharmaceutical Compositions

In some embodiments, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of one or a plurality of the antigen binding proteins of the invention together with a pharmaceutically effective diluents, carrier, solubilizer, emulsifier, preservative, and/or adjuvant. In certain embodiments, the antigen binding protein is an antibody, including a drug-conjugated antibody or a bispecific antibody. Pharmaceutical compositions of the invention include, but are not limited to, liquid, frozen, and lyophilized compositions.

Preferably, formulation materials are nontoxic to recipients at the dosages and concentrations employed. In specific embodiments, pharmaceutical compositions comprising a therapeutically effective amount of a CD27L antigen binding protein, e.g, a CD27L-binding ADC, are provided.

In certain embodiments, the pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In such embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, proline, or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA));

complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. See, REMINGTON'S PHARMACEUTICAL SCIENCES, 18" Edition, (A. R. Genrmo, ed.), 1990, Mack Publishing Company.

In certain embodiments, the optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. See, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, supra. In certain embodiments, such compositions may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of the antigen binding proteins of the invention. In certain embodiments, the primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. In specific embodiments, pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, and may further include sorbitol or a suitable substitute therefor. In certain embodiments of the invention, CD27L antigen binding protein compositions may be prepared for storage by mixing the selected composition

having the desired degree of purity with optional formulation agents (REMINGTON'S PHARMACEUTICAL SCIENCES, supra) in the form of a lyophilized cake or an aqueous solution. Further, in certain embodiments, the CD27L antigen binding protein product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

The pharmaceutical compositions of the invention can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. Preparation of such pharmaceutically acceptable compositions is within the skill of the art. The formulation components are present preferably in concentrations that are acceptable to the site of administration. In certain embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be provided in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired CD27L antigen binding protein in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which the CD27L antigen binding protein is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that may provide controlled or sustained release of the product which can be delivered via depot injection. In certain embodiments, hyaluronic acid may also be used, having the effect of promoting sustained duration in the circulation. In certain embodiments, implantable drug delivery devices may be used to introduce the desired antigen binding protein.

Pharmaceutical compositions of the invention can be formulated for inhalation. In these embodiments, CD27L antigen binding proteins are advantageously formulated as a dry, inhalable powder. In specific embodiments, CD27L antigen binding protein inhalation solutions may also be formulated with a propellant for aerosol delivery. In certain embodiments, solutions may be nebulized. Pulmonary administration and formulation methods therefore are further described in International Patent Application

No. PCT/US94/001875, which is incorporated by reference and describes pulmonary delivery of chemically modified proteins.

It is also contemplated that formulations can be administered orally. CD27L antigen binding proteins that are administered in this fashion can be formulated with or without carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. In certain embodiments, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the CD27L antigen binding protein. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving CD27L antigen binding proteins in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See, for example, International Patent Application No. PCT/US93/00829, which is incorporated by reference and describes controlled release of porous polymeric microparticles for delivery of pharmaceutical compositions. Sustained-release preparations may include semipermeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (as disclosed in U.S. Pat. No. 3,773,919 and European Patent Application Publication No. EP 058481, each of which is incorporated by reference), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 1983, Biopolymers 2:547-556), poly (2-hydroxyethyl-methacrylate) (Langer et al., 1981, J. Biomed. Mater. Res. 15:167-277 and Langer, 1982, Chem. Tech. 12:98-105), ethylene vinyl acetate (Langer et al., 1981, supra) or poly-D(-)-3-hydroxybutyric acid (European Patent Application Publication No. EP 133,988). Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art. See, e.g., Eppstein et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82:3688-3692; European

Patent Application Publication Nos. EP 036,676; EP 088,046 and EP 143,949, incorporated by reference.

Pharmaceutical compositions used for in vivo administration are typically provided as sterile preparations. Sterilization can be accomplished by filtration through sterile filtration membranes. When the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. Compositions for parenteral administration can be stored in lyophilized form or in a solution. Parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Aspects of the invention includes self-buffering CD27L antigen binding protein formulations, which can be used as pharmaceutical compositions, as described in international patent application WO 06138181A2 (PCT/US2006/022599), which is incorporated by reference in its entirety herein.

As discussed above, certain embodiments provide CD27L antigen binding proteins protein compositions, particularly pharmaceutical CD27L antigen binding protein compositions, that comprise, in addition to the CD27L antigen binding protein, one or more excipients such as those illustratively described in this section and elsewhere herein. Excipients can be used in the invention in this regard for a wide variety of purposes, such as adjusting physical, chemical, or biological properties of formulations, such as adjustment of viscosity, and or processes of the invention to improve effectiveness and or to stabilize such formulations and processes against degradation and spoilage due to, for instance, stresses that occur during manufacturing, shipping, storage, pre-use preparation, administration, and thereafter.

A variety of expositions are available on protein stabilization and formulation materials and methods useful in this regard, such as Arakawa et al., "Solvent interactions in pharmaceutical formulations," Pharm Res. 8(3): 285-91 (1991); Kendrick et al., "Physical stabilization of proteins in aqueous solution," in: RATIONAL DESIGN OF STABLE PROTEIN FORMULATIONS: THEORY AND PRACTICE, Carpenter and Manning, eds. Pharmaceutical Biotechnology. 13: 61-84 (2002), and Randolph et al., "Surfactant-protein interactions," Pharm Biotechnol. 13: 159-75 (2002), each of which is

herein incorporated by reference in its entirety, particularly in parts pertinent to excipients and processes of the same for self-buffering protein formulations in accordance with the current invention, especially as to protein pharmaceutical products and processes for veterinary and/or human medical uses.

Salts may be used in accordance with certain embodiments of the invention to, for example, adjust the ionic strength and/or the isotonicity of a formulation and/or to improve the solubility and/or physical stability of a protein or other ingredient of a composition in accordance with the invention.

As is well known, ions can stabilize the native state of proteins by binding to charged residues on the protein's surface and by shielding charged and polar groups in the protein and reducing the strength of their electrostatic interactions, attractive, and repulsive interactions. Ions also can stabilize the denatured state of a protein by binding to, in particular, the denatured peptide linkages (--CONH) of the protein. Furthermore, ionic interaction with charged and polar groups in a protein also can reduce intermolecular electrostatic interactions and, thereby, prevent or reduce protein aggregation and insolubility.

Ionic species differ significantly in their effects on proteins. A number of categorical rankings of ions and their effects on proteins have been developed that can be used in formulating pharmaceutical compositions in accordance with the invention. One example is the Hofmeister series, which ranks ionic and polar non-ionic solutes by their effect on the conformational stability of proteins in solution. Stabilizing solutes are referred to as "kosmotropic." Destabilizing solutes are referred to as "chaotropic." Kosmotropes commonly are used at high concentrations (e.g., >1 molar ammonium sulfate) to precipitate proteins from solution ("salting-out"). Chaotropes commonly are used to denture and/or to solubilize proteins ("salting-in"). The relative effectiveness of ions to "salt-in" and "salt-out" defines their position in the Hofmeister series.

Free amino acids can be used in CD27L antigen binding protein formulations in accordance with various embodiments of the invention as bulking agents, stabilizers, and antioxidants, as well as other standard uses. Lysine, proline, serine, and alanine can be used for stabilizing proteins in a formulation. Glycine is useful in lyophilization to ensure correct cake structure and properties. Arginine may be useful to inhibit protein

aggregation, in both liquid and lyophilized formulations. Methionine is useful as an antioxidant.

Polyols include sugars, e.g., mannitol, sucrose, and sorbitol and polyhydric alcohols such as, for instance, glycerol and propylene glycol, and, for purposes of discussion herein, polyethylene glycol (PEG) and related substances. Polyols are kosmotropic. They are useful stabilizing agents in both liquid and lyophilized formulations to protect proteins from physical and chemical degradation processes. Polyols also are useful for adjusting the tonicity of formulations.

Among polyols useful in select embodiments of the invention is mannitol, commonly used to ensure structural stability of the cake in lyophilized formulations. It ensures structural stability to the cake. It is generally used with a lyoprotectant, e.g., sucrose. Sorbitol and sucrose are among preferred agents for adjusting tonicity and as stabilizers to protect against freeze-thaw stresses during transport or the preparation of bulks during the manufacturing process. Reducing sugars (which contain free aldehyde or ketone groups), such as glucose and lactose, can glycate surface lysine and arginine residues. Therefore, they generally are not among preferred polyols for use in accordance with the invention. In addition, sugars that form such reactive species, such as sucrose, which is hydrolyzed to fructose and glucose under acidic conditions, and consequently engenders glycation, also is not among preferred polyols of the invention in this regard. PEG is useful to stabilize proteins and as a cryoprotectant and can be used in the invention in this regard.

Embodiments of the CD27L antigen binding protein formulations further comprise surfactants. Protein molecules may be susceptible to adsorption on surfaces and to denaturation and consequent aggregation at air-liquid, solid-liquid, and liquid-liquid interfaces. These effects generally scale inversely with protein concentration. These deleterious interactions generally scale inversely with protein concentration and typically are exacerbated by physical agitation, such as that generated during the shipping and handling of a product.

Surfactants routinely are used to prevent, minimize, or reduce surface adsorption. Useful surfactants in the invention in this regard include polysorbate 20, polysorbate 80, other fatty acid esters of sorbitan polyethoxylates, and poloxamer 188.

Surfactants also are commonly used to control protein conformational stability. The use of surfactants in this regard is protein-specific since, any given surfactant typically will stabilize some proteins and destabilize others.

Polysorbates are susceptible to oxidative degradation and often, as supplied, contain sufficient quantities of peroxides to cause oxidation of protein residue side-chains, especially methionine. Consequently, polysorbates should be used carefully, and when used, should be employed at their lowest effective concentration. In this regard, polysorbates exemplify the general rule that excipients should be used in their lowest effective concentrations.

Embodiments of CD27L antigen binding protein formulations further comprise one or more antioxidants. To some extent deleterious oxidation of proteins can be prevented in pharmaceutical formulations by maintaining proper levels of ambient oxygen and temperature and by avoiding exposure to light. Antioxidant excipients can be used as well to prevent oxidative degradation of proteins. Among useful antioxidants in this regard are reducing agents, oxygen/free-radical scavengers, and chelating agents. Antioxidants for use in therapeutic protein formulations in accordance with the invention preferably are water-soluble and maintain their activity throughout the shelf life of a product. EDTA is a preferred antioxidant in accordance with the invention in this regard.

Antioxidants can damage proteins. For instance, reducing agents, such as glutathione in particular, can disrupt intramolecular disulfide linkages. Thus, antioxidants for use in the invention are selected to, among other things, eliminate or sufficiently reduce the possibility of themselves damaging proteins in the formulation.

Formulations in accordance with the invention may include metal ions that are protein co-factors and that are necessary to form protein coordination complexes, such as zinc necessary to form certain insulin suspensions. Metal ions also can inhibit some processes that degrade proteins. However, metal ions also catalyze physical and chemical processes that degrade proteins.

Magnesium ions (10-120 mM) can be used to inhibit isomerization of aspartic acid to isoaspartic acid. Ca^{+2} ions (up to 100 mM) can increase the stability of human deoxyribonuclease. Mg^{+2}, Mn^{+2}, and Zn^{+2}, however, can destabilize rhDNase. Similarly,

Ca^{+2} and Sr^{+2} can stabilize Factor VIII, it can be destabilized by Mg^{+2}, Mn^{+2} and Zn^{+2}, Cu^{+2} and Fe^{+2}, and its aggregation can be increased by Al^{+3} ions.

Embodiments of the CD27L antigen binding protein formulations further comprise one or more preservatives. Preservatives are necessary when developing multi-dose parenteral formulations that involve more than one extraction from the same container. Their primary function is to inhibit microbial growth and ensure product sterility throughout the shelf-life or term of use of the drug product. Commonly used preservatives include benzyl alcohol, phenol and m-cresol. Although preservatives have a long history of use with small-molecule parenterals, the development of protein formulations that includes preservatives can be challenging. Preservatives almost always have a destabilizing effect (aggregation) on proteins, and this has become a major factor in limiting their use in multi-dose protein formulations. To date, most protein drugs have been formulated for single-use only. However, when multi-dose formulations are possible, they have the added advantage of enabling patient convenience, and increased marketability. A good example is that of human growth hormone (hGH) where the development of preserved formulations has led to commercialization of more convenient, multi-use injection pen presentations. At least four such pen devices containing preserved formulations of hGH are currently available on the market. Norditropin (liquid, Novo Nordisk), Nutropin AQ (liquid, Genentech) & Genotropin (lyophilized--dual chamber cartridge, Pharmacia & Upjohn) contain phenol while Somatrope (Eli Lilly) is formulated with m-cresol.

Several aspects need to be considered during the formulation and development of preserved dosage forms. The effective preservative concentration in the drug product must be optimized. This requires testing a given preservative in the dosage form with concentration ranges that confer anti-microbial effectiveness without compromising protein stability.

As might be expected, development of liquid formulations containing preservatives are more challenging than lyophilized formulations. Freeze-dried products can be lyophilized without the preservative and reconstituted with a preservative containing diluent at the time of use. This shortens the time for which a preservative is in contact with the protein, significantly minimizing the associated stability risks. With

liquid formulations, preservative effectiveness and stability should be maintained over the entire product shelf-life (.about.18 to 24 months). An important point to note is that preservative effectiveness should be demonstrated in the final formulation containing the active drug and all excipient components.

CD27L antigen binding protein formulations generally will be designed for specific routes and methods of administration, for specific administration dosages and frequencies of administration, for specific treatments of specific diseases, with ranges of bio-availability and persistence, among other things. Formulations thus may be designed in accordance with the invention for delivery by any suitable route, including but not limited to orally, aurally, opthalmically, rectally, and vaginally, and by parenteral routes, including intravenous and intraarterial injection, intramuscular injection, and subcutaneous injection.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, crystal, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) that is reconstituted prior to administration. The invention also provides kits for producing a single-dose administration unit. The kits of the invention may each contain both a first container having a dried protein and a second container having an aqueous formulation. In certain embodiments of this invention, kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes) are provided.

The therapeutically effective amount of a CD27L antigen binding protein-containing pharmaceutical composition to be employed will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will vary depending, in part, upon the molecule delivered, the indication for which the CD27L antigen binding protein is being used, the route of administration, and the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient. In certain embodiments, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 μg/kg to up to about 30 mg/kg or more, depending on the factors mentioned above. In specific

embodiments, the dosage may range from 1.0 μg/kg up to about 20 mg/kg, optionally from 10 μg/kg up to about 10 mg/kg or from 100 μg/kg up to about 5 mg/kg.

A therapeutic effective amount of a CD27L antigen binding protein preferably results in a decrease in severity of disease symptoms, in increase in frequency or duration of disease symptom-free periods or a prevention of impairment or disability due to the disease affliction. For treating CD27L-expressing tumors, a therapeutically effective amount of CD27L antigen binding protein, e.g. an anti-CD27L ADC, preferably inhibits cell growth or tumor growth by at least about 20%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% relative to untreated patients. The ability of a compound to inhibit tumor growth may be evaluated in an animal model predictive of efficacy in human tumors.

Pharmaceutical compositions may be administered using a medical device. Examples of medical devices for administering pharmaceutical compositions are described in U.S. Patent Nos. 4,475,196; 4,439,196; 4,447,224; 4,447, 233; 4,486,194; 4,487,603; 4,596,556; 4,790,824; 4,941,880; 5,064,413; 5,312,335; 5,312,335; 5,383,851; and 5,399,163, all incorporated by reference herein.

Methods of Diagnosing or Treating a CD27L-associated Disease or Disorder

The CD27L antigen binding proteins of the invention are particularly useful for detecting CD27L in a biological sample. In certain embodiments, a biological sample obtained from a patient is contacted with a CD27L antigen binding protein. Binding of the CD27L antigen binding protein to CD27L is then detected to determine the presence or relative amount of CD27L in the sample. Such methods may be useful in diagnosing or determining patients that are amenable to treatment with a CD27L antigen binding protein, e.g., an anti-CD27L ADC.

In certain embodiments, a CD27L antigen binding protein of the invention is used to diagnose, detect, or treat an autoimmune or inflammatory disorder. In treating autoimmune or inflammatory disorders, the CD27L antigen binding protein may target CD27L-expressing cells of the immune system for destruction and/or may block the interaction of CD27L with the receptor CD27.

CD27L interaction with CD27 is thought to play a role in cell-mediated autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) (Nakajima et al. (2000) J. Neuroimmunol. 109:188-96). This effect is thought to be mediated in part by an inhibition of TNF-a production. Furthermore, blocking of CD27L signaling inhibits CD40-mediated clonal expansion of CD8+ T-cells and reduces the generation of CD8+ memory T-cells (Taraban et al. (2004) J. Immunol. 173:6542-6). As such, the CD27L antigen binding proteins can be used to treat a subject with an autoimmune disorder, e.g., a disorder characterized by the presence of B-cells expressing CD27L including, for example, experimental autoimmune encephalomyelitis. Additional autoimmune disorders in which the antibodies of this disclosure can be used include, but are not limited to systemic lupus erythematosus (SLE), insulin dependent diabetes mellitus (IDDM), inflammatory bowel disease (IBD) (including Crohn's Disease, ulcerative colitis and Celiac disease), multiple sclerosis (MS), psoriasis, autoimmune thyroiditis, rheumatoid arthritis (RA) and glomerulonephritis. Furthermore, the cd27L antigen binding protein compositions of this disclosure can be used for inhibiting or preventing transplant rejection or in the treatment of graft versus host disease (GVHD).

Additionally, the interaction of CD27L with CD27 has also been proposed to play a role in signaling on CD4+ T cells. Some viruses have been shown to signal the CD27 pathway, leading to destruction of neutralizing antibody responses (Matter et al. (2006) J Exp Med 203:2145-55). As such, the CD27L antigen binding protein compositions and methods of the present disclosure can be used to treat a subject with a viral infection including, for example, infections from human immunodeficiency virus (HIV), Hepatitis (A, B, & C), Herpesvirus, (e.g., VZV, HSV-1, HAV-6, HSV-II and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus and lymphocytic choriomeningitis virus (LCMV) or in the treatment of HIV infection/AIDS. Additionally, the human antibodies, antibody compositions and methods of the present disclosure can be used to inhibit TNF-a production.

In certain embodiments, a CD27L antigen binding protein of the invention is used to diagnose, detect, or treat a cancer or tumorigenic disorder. Tumors and cancers amenable for treatment herein, wherein the cells of the tumor or cancer may express CD27L include, but are not limited to: renal cell carcinomas (RCC), such as clear cell RCC, papillary RCC, chromophobe RCC,and the like, glioblastoma, Head and Neck Cancers (e.g., squamous cell carcinomas(HNSCC), and the like), breast cancer, brain tumors, nasopharangeal carcinomas, non-Hodgkin's lymphoma (NHL), such as low grade NHL, diffuse large cell NHL, and the like, acute lymphocytic leukemia (ALL), such as pre-B-ALL, and the like, chronic lymphocytic leukemia (CLL or B-CLL), Burkitt's lymphoma, anaplastic large-cell lymphomas (ALCL), multiple myeloma, cutaneous T-cell lymphomas, nodular small cleaved-cell lymphomas, lymphocytic lymphomas, peripheral T-cell lymphomas, Lennert's lymphomas, immunoblastic lymphomas, T-cell leukemia/lymphomas (ATLL), adult T-cell leukemia (T-ALL), entroblastic/centrocytic (cb/cc) follicular lymphomas cancers, diffuse large cell lymphomas of B lineage, angioimmunoblastic lymphadenopathy (AILD)-like T cell lymphoma, HIV associated body cavity based lymphomas, embryonal carcinomas, undifferentiated carcinomas of the rhino-pharynx (e.g., Schmincke's tumor), Castleman's disease, Kaposi's Sarcoma, multiple myeloma, Waldenstrom's macroglobulinemia and other B-cell lymphomas. Additional tumor types amenable for treatment herein, include tumors of the: kidney, pancreas, larynx or pharynx, melanoma, ovary, lung adenocarcinoma, colon breast, brain, and the like.

EXAMPLES

The following examples, both actual and prophetic, are provided for the purpose of illustrating specific embodiments or features of the present invention and are intended to limit its scope.

Example 1 -- Fully human monoclonal antibodies against CD27L

The generation of fully human antibodies directed against human CD27L was carried out using XENOMOUSE® technology (United States Patent Nos. 6,114,598; 6,162,963; 6,833,268; 7,049,426; 7,064,244, which are incorporated herein by reference

in their entirety; Green *et al.*, 1994, *Nature Genetics* 7:13-21; Mendez *et al.*, 1997, *Nature Genetics* 15:146-156; Green and Jakobovitis, 1998, *J. Ex. Med.* 188:483-495).

IgG1, IgG2, and IgG4 XENOMOUSE® mice were immunized/boosted with soluble human CD27L or human CD27L recombinantly expressed on the surface of Chinese Hamster Ovary (CHO) cells. Hybridomas were produced from the immunized mice. Hybridoma supernatants were screened for binding to recombinant human CD27L-expressing 293 cells. Over 260 supernatants were positive for binding.

The positive supernatants were then screened for binding to native CD27L on the surface of Raji and/or 786-0 cells. The native CD27L binding assay revealed 161 positive supernatants.

Those supernatants were tested for cross-reactivity with cynomolgus CD27L. Twenty-five supernatants were positive for cross-reactivity with cynomolgus monkey CD27L. Those twenty five were then tested for CDC activity and the ability to block human CD27 binding to human CD27L. Nineteen supernatants were positive for CDC activity and for inhibition of CD27 binding to CD27L. Subcloning and sequencing of the 13 IgG1 and 6 IgG4 lines revealed 7 unique IgG1 antibodies (Ab1-Ab7) and 1 unique IgG4 antibody (Ab8).

A summary of the characteristics of the eight antibodies along with those of a chimeric version of a commercially available mouse anti-human CD27L antibody are provided in FIG. 1.

Affinity

Affinity for recombinant soluble human CD27L was determined using a CM5 chip on a BIACORE 3000. Goat anti-human IgG antibody was used to capture the test antibody. Binding and dissociation of Histidine-tagged human soluble CD27L was measured to determine K_a, K_d, and K_D. The conditions were as follows:

Temperature = 25°C

Flow Rate = 50 ul/min

Running Buffer = HBS-EP

Regenerations with 10mM Glycine pH 1.5

Concentration range of Hu CD27L-his were 200 nM → 0.217 nM

Model Fit (Scrubber2): 1:1 Binding + local Rmax

5 Minute Association and 25 Minute Dissociation

ADCC Activity

The ADCC assay was performed in a sterile 96 well round bottom tissue culture plate (Corning). Antibodies were titrated from 20 μg/mL to 0.0002 μg/mL by carrying 10 μL in 100μL of complete RPMI containing 10% FCS (a 1:10 dilution). Calcein-labeled targets were added, 50 μL to contain 10,000 cells. Target cells and various concentrations of antibody were incubated for 40 minutes at 4° C, then NK cell effectors added, 50 μL to contain 200,000 cells. Cultures were incubated for 4 hrs at 37° C then supernatants pulled and assayed for calcein release by measuring fluorescence at 485-535 nm on a Wallac Victor II 1420 Multilable HTS counter. 100% lysis values were determined by lysing six wells of calcein labeled Raji targets with Igepal 630 detergent (3 μL per well) and spontaneous lysis values determined by measuring the fluorescence in supernatants from targets alone.

Percent (%) specific lysis was defined as (sample fluorescence) – (spontaneous lysis fluorescence)/(100% lysis – spontaneous lysis fluorescence). Raw data was entered in an Excel spreadsheet with the embedded formulae to calculate % specific lysis and resultant values transferred to graphic program (GraphPad Prism) where the data was transformed in a sigmoidal curve fit graph. Subsequent analyses (linear regression calculations) were done in GraphPad to generate EC_{50} values.

ADCP activity

Monocytes were negative selected from human peripheral blood and stored in 4°C cold room over night with medium RPMI 1640 containing 10% FBS. Then monocytes were seeded to a 48-well tissue culture plate at 200,000 cells per well with 200 μL of growth medium (RPMI 1640 containing 10% FBS and 40 ng/ml Hu M-CSF) and incubated at 37°C, 5% CO_2 for 6 days to let monocytes differentiate to macrophages.

On Day 6, the ADCP assay was performed as follows:

1. Labeling target cells with PKH67 green dye at final concentrations of $2x10^{-6}$ M PKH67 dye

- Tumor cells were collected and washed once with PBS by centrifuging the cells (400 ´ g) for 5 minutes.

- After centrifuging cells, the supernatant was carefully aspirated, but leaving no more than 25 mL of supernatant.
- Four µL of the PKH67 ethanolic dye solution at stock concentration of $4x10^{-6}$ M was added to 1 ml of Diluent C from kit in polypropylene tube and mixed well.
- Cell pellets were re-suspended into 1 mL of Diluent C at a density of 20×10^6 in polypropylene tube.
- Cells were rapidly transferred to dye work solution with gently pipetting to insure complete dispersion.
- The mixture was incubated at room temperature for 4 minutes with mixing periodically.
- Two mL of whole activated FBS was added into cells to stop the staining and incubated at room temp for 1 minute to allow binding of excess dye.
- Forty mL of RPMI containing 10%FBS was added into cells and washed once by centrifuging the cells (400 ´ g) for 10 minutes.
- Cell pellets were suspended with 40 mL of medium again and transferred to a new tube.
- Cells were washed again three times with medium RPMI +10% FBS and 1x with complete growth medium (RPMI 1640 containing 10% FBS and 40 ng/ml Hu M-CSF).
- Cells were counted and suspended with growth medium at $1x10^6$ cells per mL for T:E at 1:2 ratio.

2. Treatment of tumor cells with antibodies for antibody dependent cellular phagocytosis (ADCP)
    - Antibody dilutions were prepared in macrophage growth medium. These dilutions were concentrated at four times higher than final concentrations.
    - To preincubate PKH67 green labeled target cells with antibodies, 280 ul of 4x concentrated antibodies was mixed with 280 ul of green labeled tumor cells and incubated at 4°C for 30 minutes.
    - The mixture of green labeled tumor cells with anti-tumor antibodies was added to macrophage cells in 48-well plate at 200 µl for each well as

indicated in the Experiment Design table below. The final volume is 0.4 ml per well. The ratio of target cells to effect cells (macrophages) is 1:2.

- Cells were incubated at 37oC, 5% CO_2 for one hour.

3. Counterstaining macrophages with macrophages marker
   - Target cells and macrophages in 48-well plate were detached with Trypsin-Versene mixture.
   - Cells were transferred into a 96-well block with 2.2-ml volume per well and washed once with pre-warmed FASC wash solution by spinning the blocks at 400 ′ g for 5 minutes and then discarding supernatant.
   - Macrophages were stained with their marker, CD11b-Biotin at 1:200 dilution in block solution with 100 µl per well for 10 min on ice.
   - After washing cells once, macrophages were detected with streptavidin Alexa 568 at 1:1000 dilutions for 10 minute on ice.
   - After washing cells 1x with PBS, cells were fixed with 4% formaldehyde-PBS at room temperature for 20 minutes. Then cells were washed 1x with dH2O.
   - Cell pellets were resuspended with water at 200 µl per well and transferred to a 96-well plate at 100 µl per well.
4. Quantitative measurement of phagocytosis activity on an ArrayScan V^{TI} HCS reader (version 6, Cellomics Inc. Thermo Fisher Scientific, Pittsburgh, PA) with Target Activation BioApplication employing a 20x objective. The filter setting was indicated in Table 2. At least 200 cells were counted in each well.

Table 2

Channel	Target	Label	Fluor
1	macrophages	Ms-anti-Hu CD11b Biotin→streptavidin Alexa 568	red
2	Tumor cells	PHK67	green

Statistical analysis was performed using Prism 4.01 (GraphPad, San Diego, CA). A plot shows % tumor cell phagocytosis vs. the log of antibody concentration in ng/ml.

The percentage of tumor cell phagocytosis is represented with percentage of tumor cells that were overlapped with macrophages vs. total macrophages in the selected fields and obtained from output feature of ArrayScan reader "%ObjectCounts". The % values were expressed as the mean +/- standard error of the mean (SEM) for duplicate measurements (n = 2). The EC_{50} was determined by using nonlinear regression (curve fit) followed by using Sigmoidal dose-response equation. Data were normalized to the maximum and minimum signal and fit to a sigmoidal dose-response curve.

CDC activity

Preparation of tumor cells: Raji cells were washed once with assay medium and resuspended in assay medium (RPMI 1640 plus 1% FBS). Cells were seeded in a 96-well tissue culture plate at 50 µL per well with two cell densities for both rabbit and human complement complements. For rabbit complement, the cell density was $5x10^4$cells per well. For human complement, the cell density was $2x10^5$ cells per well.

Treatment of cells with complement and test antibody: Three times concentrated complements were prepared in assay medium as outlined in Table 3. Then they were added to cells in plates at 50 µL per well. For cells treated with rabbit complement, the final concentration of rabbit complement was 10%; for cells treated with human complement, the final concentration of human complement was 20%.

Table 3: Preparation of 3x concentrated complement in assay medium (RPMI 1640+1%FBS)

3x Complement	Complement (ml)	Assay Medium (ml)	Total Volume (ml)
30% HI rabbit C' (inactive)	0.5	1.16	1.66
30% no HI rabbit C' (active)	2.5	5.83	8.33
60% HI Hu C' (inactive)	0.5	0.33	0.83
60% no HI Hu C' (active)	3	2.0	5.0

Test antibodies at 10 µg/mL were added to cells at 50 µL per well. The total volume in each well at the start of culture was 150 µL. Cells were continuously incubated

at 37°C, 5% CO_2 for one hour for cells treated with rabbit complement and 6 hours for cells treated with human complement.

Measurement of cytotoxicity with ArrayScan plate reader: After incubation, 50 µl medium from each well was removed. The cocktail of Hoechst 33342 and propidium iodide (PI) which was prepared at 1:1000 dilution in PBS solution containing 2% FBS was added into cells at 100 µL per well. Cells treated with human complement were transferred into a new 96-well plate at 40 µL per well after gently mixing. Samples were analyzed on an ArrayScan V^{TI} HCS reader (version 6, Cellomics, Thermo Fisher Scientific, Pittsburgh, PA) with BioApplication "Target Activation" employing a 20x objective. The filter setting was indicated in Table 4. At least 200 cells were counted in each well.

Table 4

Channel	Target	Label	Fluor	Filter
1	Nucleic acid for all cells	Hoechst 33342	UV/460 nm	DAPI
2	Nucleic acid for dead cells	Propidium iodide	488/>575 nm	TRITC

Statistical Analysis: Statistical analysis was performed using Prism 4.01 (GraphPad, San Diego, CA).

Internalization Time

Seeding cells: 786-0 cells were seeded on a 96-well plate at 10,000 cells per well with 100 µL of growth medium (RPMI containing 10% FBS) and incubated at 37°C, 5% CO_2 for 2 days to reach to 100% cell confluency on assay day. Plated cells were evaluated for 1) internalization and 2) endosomal co-localization.

Staining cells for internalization time-course: Plate was washed 1x with assay medium (PBS containing 2% FBS). Antibodies were added to wells at 2 µg/mL per well (100 µL per well) in assay medium. Human antibodies were allowed to bind to cells at 4°C for 30 minutes and then washed 1x with assay medium. Anti-human IgG Fab' Alexa 488 (1:100) and Hoechst 33342 (1:2000) were added to cells in assay medium at 4°C for

20 minutes. Next cells were washed 1x with assay medium. Cells were either fixed and permeabilized, starting with time zero or incubated at 37°C, 5% CO_2 for 1, 3, or 5 hours. Post incubation, cells were fixed and permeabilized with the following procedure. Cells were washed 1x with BD wash buffer, followed by addition of Fix/perm solution to wells at 100 µL per well. Samples were analyzed on an ArrayScan V^{TI} HCS reader (version 6, Cellomics, Thermo Fisher Scientific, Pittsburgh, PA) with BioApplication "Spot Detector" employing a 40x objective. The filter setting for internalization was indicated in Table 5. At least 400 cells were counted in each well.

Table 5

Channel	Target	Label	Filter
1	Nucleic acid for all cells	Hocchst 33342	DAPI (blue)
2	Internalized spots	Alexa 488	FITC (green)

Count-staining cells for co-localizing internalized spots to the early endosomal compartment: After internalization analysis, 786-0 cells were washed 1x with BD buffer. Cells were explored to Fix/perm solution at RT for 20 minutes. Following 2x wash steps, cells were incubated with EEA-1 in BD buffer at 0.5 µg/mL (100 µl/well) at RT for 20 minutes. Cells were washed 1x with BD buffer and added with anti-mouse Alexa 568 at 1:1000 dilution. Cells were incubated at RT for 20 minutes followed by two wash steps of BD buffer solution.

Photographing images: Cell images for internalization and co-localization were taken with a Leica florescent microscope connected to Hamamutsu digital camera with Openlab Image Analysis software (Improvision Inc, Lexington, MA) or ArrayScan V^{TI} HCS reader.

Statistical Analysis: Statistical analysis was performed using Prism 4.01 (GraphPad, San Diego, CA). The spot counts were expressed as the mean ± standard error of the mean (SEM) for duplicate measurements (n = 2). Using analysis tool, spot count per unit time (internalization rate) was fit to a one phase exponential association equation.

Example 2 – Assessment of MCC-DM1-conjugated antibodies

Ab1, Ab2, Ab4, Ab7, and Ab8 were conjugated to MCC-DM1 (see Figure 12). The targeted load level was 4.5-5 drugs per antibody. Briefly, the lysines of the antibody were conjugated to the NHS-ester of the hetero-bifunctional cross-linker Succinimidyl 4-[*N*-maleimidomethyl] cyclohexane-1-carboxylate (SMCC) which contains an NHS-ester and a maleimide. Next the linker-modified antibody was purified from excess linker and then conjugated to the warhead DM1 via a sulfhydryl present on DM1. Excess DM1 was then removed by a second purification step to generate the final conjugate Ab-MCC-DM1.

More particularly, CD27L antibody, transiently expressed in mammalian cell culture 2936-E cells, was loaded onto a MabSelect SuRe column (GE Healthcare) that had been equilibrated in 25mM Tris, 150mM Sodium Chloride, pH 7.4. The column with bound CD27L antibody was then washed with 3 wash steps: first an equilibration buffer wash, followed by a 25mM Tris, 500mM L-Arginine, pH 7.5 wash and a final wash with equilibration buffer. CD27L antibody was eluted with 100mM Sodium Acetate, pH 3.5. Fractions containing the antibody were pooled and adjusted to a final pH of 5.0 with 1M Tris, pH 8.0. The antibody was subsequently purified on a Fractogel® EMD SO3-(M) column (EMD Chemicals Inc) equilibrated in 30mM Sodium Acetate, pH 5.0. Bound antibody was eluted with an 8CV gradient between 0 to 0.8M Sodium Chloride in 30mM Sodium Acetate, pH 5.0. Antibody containing fractions were pooled and dialyzed into Conjugation Buffer (2mM EDTA, 50mM Sodium Chloride, 50mM Potassium Phosphate, pH 6.5).

The purified CD27L antibody was modified with the amine reactive linker Succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) (Thermo Scientific) to introduce thiol reactive maleimide groups. The antibody at a concentration of 55μM was treated with 20 molar equivalents of SMCC in Conjugation Buffer adjusted to 10% dimethylacetamide (v/v) in the final reaction mixture. After incubation for 90 minutes at room temperature, the reaction mixture was desalted with a HiPrep 26/10 Desalting Column containing Sephadex G-25 fine resin (GE Healthcare) equilibrated in 2mM EDTA, 150mM Sodium Chloride, 35mM Sodium Citrate, pH 5.0. Antibody containing fractions were pooled and assayed for degree of modification with linker using

Ellman's reagent (5,5'-dithio-bis-[2-nitrobenzoic acid]) as described below. The antibody was found to be modified with an average of 7.5 maleimide groups per antibody.

Ellman's reagent is cleaved by thiols, yielding a yellow product with an absorbance at 412nm. A subtractive Ellman's assay was used to determine the number of maleimide groups on the CD27L antibody after reaction with SMCC. CD27L antibody modified with SMCC or a control sample of buffer without antibody was incubated with an equivalent concentration of thiols, 0.4mM DTT (dithiothreitol). Any maleimides present in the antibody sample would react with the thiols in DTT, making it unavailable for further reaction with Ellman's reagent. Ellman's reagent was then added to both samples and a colorimetric quantitation at 412nm was made to determine the concentration of reacted Ellman's reagent. The decreased concentration of thiols in the antibody sample as compared to the control sample is proportional to the number of maleimides present in the antibody sample. This value was used to determine the number of linked maleimide groups per modified CD27L antibody.

The SMCC modified CD27L antibody (7.5 maleimide groups per antibody) at a concentration between 17μM - 27μM was treated with 1.7 molar equivalents of DM1 (Immunogen) per maleimide group buffered with 2mM EDTA, 150mM NaCl, 35mM Sodium Citrate, pH 5.0 adjusted to 3% DMA (v/v) in the final reaction mixture. Reaction mixtures were incubated at room temperature overnight for up to 20 hours. The reaction mixture was loaded on a Superdex 200 gel filtration column (GE Healthcare) equilibrated with 20mM Sodium Phosphate, 150 mM Sodium Chloride, pH 6.5. Fractions were collected and and monomeric antibody containing fractions pooled and assayed. The molar ratio of DM1 molecules linked per antibody was determined by measuring the absorbance at 252 nm and 280 nm, and found to be 4.5 – 5.0 DM1 molecules per antibody.

In vitro pharmacology

The antibody drug conjugates (ADC) Ab4- and Ab8-MCC-DM1 demonstrated comparable native CD27L binding to their un-conjugated counterparts as assessed by flow cytometry. The observed binding EC50 for Ab4 and Ab4-MCC-DM1 were the same while it was a modest 4-fold lower for Ab8-MCC-DM1 as compared to Ab8 (Table

6). A more precise measure of binding affinity was determined for Ab4, Ab8 and their conjugate counterparts by measuring their ability to bind native human CD27L expressed on Raji cells using Gyros technology. The results showed that both conjugates exhibited sub-nM affinities for CD27L within two-fold of those observed for the un-conjugated Ab4 and Ab8 (Table 6). Both unconjugated and conjugated Ab4 and Ab8 also exhibited binding affinities to soluble human CD27L-his within 2-fold of each other as determined by BIACORE.

Table 6. Binding comparison of Ab4 and Ab8 to their MCC-DM1 conjugate counterparts

Measurement	Ab4	Ab4-MCC-DM1	Ab8	Ab8-MCC-DM1
Gyros K_D—native CD27L-Raji	0.014 nM	0.023 nM	0.078 nM	0.077 nM
FACS (EC_{50})—786-0	0.1 nM	0.1 nM	0.02 nM	0.08 nM

The internalization of Ab4-MCC-DM1, Ab8-MCC-DM1, Ab4, and Ab8 was evaluated in the CD27L-expressing human ccRCC line 786-0. CD27L expressing 786-0 cells were exposed to un-conjugated Ab4 and Ab8 human anti-CD27L antibody, Ab4-MCC-DM1, Ab8-MCC-DM1, control HuIgG1, or control αSA-MCC-DM1 and allowed to bind at 4°C. Internalization of the test articles was evaluated using FluoroNanogold goat anti-hu IgG Fab' Alexa 488. Cells were fixed and permeabilized at specific times after incubation with test articles (time = 0, 1, 3, and 5 hour) and imaged using an ArrayScan VTI HCS reader. Co-localization of internalized test articles to endosomes was determined using anti-EEA-1 antibody, an endosomal marker. The time-dependent internalization of the antibodies, including Ab4-MCC-DM1, was observed via fluorescence microscopy image analysis by the formation of punctate spots within the cellular cytoplasm at 37°C compared with cell membrane localization at time zero at 4°C. Ab4-MCC-DM1 co-localized with the endosomal marker EEA-1 after a 5-hour incubation at 37°C, demonstrating that Ab4-MCC-DM1 is internalized into the endosomal subcellular compartment of 786-0 cells. The level and rate of internalization was within a similar range for Ab4, Ab8 and their conjugated counter-parts (Figure 2).

Both Ab4-MCC-DM1 and Ab8-MCC-DM1 demonstrated potent and specific in vitro growth inhibition of CD27L-expressing tumor cells. In an anti-proliferation (tumor growth inhibition) assay, Ab4-MCC-DM1, Ab8-MCC-DM1 or un-conjugated anti-

CD27L Ab4 or Ab8 were incubated with CD27L expressing 786-0 luciferase or CD27L negative H1650 target cells in the presence/absence of naked Ab4 or Ab8, respectively, or control HuIgG1 for 4 days. Both cell lines, 786-0 luciferase and H1650, were seeded in 96-well tissue culture plates with growth medium at 100 µL of well with 500 cells per well for 786-0 luciferase and 1000 cells per well for H1650. All plates were incubated at 37oC, 5% CO2 for 4 hours. After a 4 hour incubation, naked Ab and conjugates were added to cells at 100 µL per well at various dose titrations. The total volume in each well at the start of culture was 200 µL. Cells were continuously incubated at 37oC, 5% CO2 for 4 days prior to measurement of cellular ATP levels. To assess cell growth inhibition, ATP levels (as a measure of cell number) were measured via luminescence using the CellTiter-Glo assay kit.

Both Ab4-MCC-DM1 and Ab8-MCC-DM1 inhibited cell growth of CD27L-expressing 786-0 cells with a concentration of 50% inhibition (IC50) as set forth in Table 7. Cell growth inhibition was not observed in CD27L-negative H1650 cells upon exposure to Ab4-MCC-DM1 and Ab8-MCC-DM1. Addition of an excess of unconjugated parental anti-CD27L antibody was able to block Ab4-MCC-DM1 mediated growth inhibition, confirming CD27L binding by Ab4-MCC-DM1 was required for activity. Unconjugated parental anti-CD27L antibody did not inhibit the growth of CD27L-expressing 786-0 cells. Control conjugate, anti-streptavidin-MCC-DM1 (αSA-MCC-DM1), did not exhibit any inhibition of cell growth in either the CD27L-positive or the CD27L-negative cell line. The results indicate that both Ab4-MCC-DM1 and Ab8-MCC-DM1 were potent inhibitors of 786-0 cell growth compared to control conjugate (Figure 3). Ab4-MCC-DM1 tended to show a slight increase in potency over Ab8-MCC-DM1 (Table 7).

Table 7. Cellular Potency of anti CD27L-MCC-DM1 conjugates

$786\text{-}0\text{-}_{IC50}$	Ab4-MCC-DM1	Ab8-MCC-DM1
Drug Conc. (nM)	0.34	0.54
Antibody Conc. (nM)	0.07	0.11

Ab4-MCC-DM1 mediated Antibody Dependent Cell Cytotoxicity (ADCC) against Raji cells with similar potency (EC50 = 0.006 μg/mL) and magnitude to that observed for the unconjugated parental Ab4 anti-CD27L antibody (EC50 = 0.01 μg/mL). Briefly, Natural Killer (NK) cells isolated from the PBMC obtained from a normal human blood donor were incubated with calcein labeled Raji human B cell lymphoma target cells (express CD27L) in the presence of Ab4-MCC-DM1 or control antibodies as described above. Percent (%) specific cytotoxicity was determined by measuring calcein release from CD27L expressing target cells lysed in the presence of Ab4-MCC-DM1 as compared to control wells. The results are shown in Figure 8 which indicate that Ab4-MCC-DM1 mediated a similar level of ADCC to that of the Ab4 antibody (EC 50 values of 0.006 and 0.01 μg/mL respectively). The huIgG1 control did not mediate any measurable lysis of CD27L expressing targets. Both the Ab4-MCC-DM1 and the unconjugated Ab4 antibody are capable of inducing NK cell mediated ADCC of CD27L specific targets at similar levels. Similar results were observed for Ab8-MCC-DM1 and the unconjugated Ab8 antibody.

vitro Antibody Dependent Cellular Phagocytosis (ADCP) activity of Ab4-MCC-DM1 or unconjugated parental antibody Ab4 was measured against both Raji and 786-0 tumor cells. Ab4-MCC-DM1 mediated a similar level of complement-mediated lysis to that observed for unconjugated parental antibody Ab4. Briefly, macrophages were differentiated from monocytes isolated from the human peripheral blood obtained from normal human blood donors. Macrophages were incubated with PHK67 green labeled CD27L expressing cell lines, 786-0 and Raji as target cells in the presence of unconjugated anti CD27L antibody Ab4, HuIgG1, Ab4-MCC-DM1, or control antibodies (αSA-MCC-DM1), as described above. Percent (%) tumor cell phagocytosis was determined from the percentage of tumor cells that were engulfed by macrophages vs. total macrophages in the selected fields. The results are shown in Figure 9 and indicate that Ab4-MCC-DM1 mediated similar ADCP potency in CD27L expressing cell lines, 786-0 and Raji. EC50 values of the unconjugated Ab4 were within 10-fold of those observed for Ab4-MCC-DM1 (see Table 8).

Table 8

	786-0	Raji
Abs	EC50 (pM)	EC50 (pM)
Ab4	0.008	0.008
Ab4-MCC-DM1	0.087	1.421

Given that the dilution curve was performed at 1:40 dilution intervals, the observed EC50 for both Ab4-MCC-DM1 and the unconjugated Ab4 are of a similar potency (with-in the margin of the dilution factor). Thus, both the conjugated Ab4-MCC-DM1 and the unconjugated WT Ab4 antibodies are capable of inducing human macrophage to mediate ADCP of CD27Lexpressing cells at similar levels. Similar results were observed for Ab8-MCC-DM1 and the unconjugated Ab8 antibody.

In vitro Complement Dependent Cytotoxicity (CDC) activity of Ab4-MCC-DM1 was assessed employing both human and rabbit complement and CD27L-expressing Raji tumor cells. At 10 μg/mL, Ab4-MCC-DM1 mediated a similar level of complement-mediated lysis to that observed for unconjugated parental antibody Ab4 (rabbit complement 72% lysis and human 17% lysis). Briefly, activated rabbit or human complements were incubated with CD27L expressing Raji target cells in the presence of anti CD27L antibodies or control antibodies, as described above. CDC mediated killing was measured from output feature of ArrayScan reader "% selected objects" to detect % of PI positive vs. Hoechst for "% Cytotoxicity." The results indicate that Ab4-MCC-DM1 mediated similar levels of CDC to that of the WT Ab4 antibody when incubating tumor Raji cells with 10% baby rabbit complement or 20% human complement. Heat inactivated rabbit or human complements didn't show any CDC mediated killing against Raji. Thus, both Ab4-MCC-DM1 and un-conjugated Ab4 induce a similar level of CDC activity against CD27L expressing tumor target cells. Similar results were observed for Ab8-MCC-DM1 and the unconjugated Ab8 antibody.

In vivo pharmacology

In vivo passaged 786-0 ccRCC cells (786-0 S4) were implanted into female CB-17/ SCID mice using growth-factor reduced MATRIGEL to generate tumor xenografts for efficacy studies. 786-0 S4 cells express an average of approximately 180,000 CD27L sites/cell. Treatment with Ab4-MCC-DM1 or Ab8-MCC-DM1 was initiated when the tumor size reached an average of approximately 250 mm^3. Tumor-bearing animals were randomized by tumor size into groups of ten animals each and dosed intravenously once. A blinded dose response study of Ab4-MCC-DM1 and Ab8-MCC-DM1 employing doses ranging from 7–64 ug DM1/kg (0.3–2.5 mg Ab/kg) was performed in this established tumor model. Robust tumor regression was observed at the low 7 ug DM1/kg, mid 25 ug DM1/kg and the high 64 ug DM1/kg doses. At the mid and high dose, complete regressions were maintained at least 28 days following a single dose (Figure 4). No body weight loss was observed in any of the dosing groups over the course of the study.

In another demonstration of in vivo efficacy, Caki-1 ccRCC cells that express an average of 59,000 CD27L sites per cell (3-fold less that 786-0 cells) were implanted into CB-17/SCID mice as described above. When the Caki-1 xenografts reached an average size of 250 mm^3, tumor-bearing animals were randomized by tumor size into groups of ten animals each and dosed intravenously once per week for 3 weeks (to more closely mimic a weekly clinical dosing regimen). A blinded dose response study of Ab4-MCC-DM1 and Ab8-MCC-DM1 employing doses ranging from 60–270 ug DM1/kg (2.4–11 mg Ab/kg) was performed in this established tumor model. Tumor regression was initially observed at the mid 120 ug DM1/kg and the high 270 ug DM1/kg doses while tumor growth inhibition was observed at the low 60ug DM1/kg dose compared to the control conjugate or vehicle. As time progressed, tumor regression in the two higher dose groups began to diminish within 7 days of receiving the last dose (Figure 5).

Like ccRCC cells, Raji B-lymphoma cells express CD27L (about 200,000 sites / cell) and internalize the anti-CD27L drug conjugate which results in mitotic arrest and cell death in vitro. Ab4-MCC-DM1 was evaluated for efficacy in the subcutaneous Raji Xenograft model. When the Raji xenografts reached an average size of 250 mm^3, tumor-bearing animals were randomized by tumor size into groups of ten animals each and dosed intravenously q4 days x 2 and then once per week for one additional week for a

total of 3 doses (to more closely mimic a weekly clinical dosing regimen). A blinded dose response study of Ab4-MCC-DM1 using doses ranging from 60–270 ug DM1/kg (2.4–11 mg Ab/kg) was performed in this established tumor model. Tumor growth inhibition was observed at all dose levels, with >90% tumor growth inhibition being observed at the mid 120 ug DM1/kg and the high 270 ug DM1/Kg doses during the active dosing period with an intermediate anti tumor response being observed at the low 60ug DM1/kg dose compared to control conjugate (Figure 6). At the end of the tumor measurement period a slight majority of animals in the mid and high dose groups exhibited tumor regression suggesting that further dose and schedule optimization could improve response.

The efficacy of different dosing intervals of control conjugate αSA-MCC-DM1, or Ab4-MCC-DM1 was evaluated in the 786-0 xenograft model. CB-17/SCID mice were implanted with 786-0 tumor cells. On day 13, 50 animals were randomized into cohorts of 10 animals each with an average tumor volume of 200 mm3. Each cohort was administered an intraperitoneal dose of either control conjugate αSA-MCC-DM1 or Ab4-MCC-DM1. Tumor volume is represented as group average ± standard error of the mean (SEM). Statistical significance of observed differences between growth curves was evaluated from days 13 to 59 by repeated measures analysis of covariance (RMANOVA) of the log transformed tumor volume data with Dunnett adjusted multiple comparisons. Significance is attained if $p < 0.05$. Three doselevels of Ab4-MCC-DM1 (1.0, 2.0, or 2.9 mg/kg of Ab4-MCC-DM1 based upon antibody or 25, 50, or 75 μg/kg based upon DM1 equivalents, respectively) were administered. The 1.0 mg/kg dose was administered once per week or once every 2 weeks for 6 weeks, the 2.0 mg/kg dose was administered once every 2 weeks for 6 weeks and the 2.9 mg/kg dose was administered once every 3 weeks for 6 weeks. Animals were dosed intraperitoneally starting on day 13 post tumor inoculation. By day 59, the αSA-MCC-DM1 treated group was euthanized due to large tumor volumes. On day 59, the average tumor volume of mice treated with each dose regimen of Ab4-MCC-DM1administered was significantly less than that of the αSA-MCC-DM1 control conjugate group ($p < 0.0001$) (Figure 7). Ab4-MCC-DM1 mediated durable tumor regression with each dosing regimen. No body weight loss was observed in any of the Ab4-MCC-DM1 treatment groups.

Pharmokinetics

Ab4 and Ab8 antibody drug conjugates were assessed after intravenous administration to female CB-17/SCID mice containing CD27L-expressing 786-0 xenografts in the context of efficacy studies and a follow-on terminal PK study. The cumulative exposures and clearance values were within 1.33 and 1.4-fold between the Ab4-MCC-DM1 and Ab8-MCC-DM1 molecules, and the half-life of both ADCs was approximately 12 days in mice.

The stability and PK parameters of the Ab4-MCC-DM1 and Ab8-MCC-DM1 antibody drug conjugates were characterized *in vivo* following a single intravenous (IV) dose into CB-17/SCID mice bearing 786-0 xenografts. The exposures of the two antibody drug conjugates were highly comparable, but Ab4-MCC-DM1 demonstrated a more stable drug-to-antibody conjugation ratio over the 96 hour time-course of the study as determined by affinity-MS than did Ab8-MCC-DM1 molecule. Ab4-MCC-DM1 showed no detectable loss of conjugate integrity at the 30 min or 24 hour time point post injection while Ab8-MCC-DM1 exhibited changes at the 30 minute time point and significant decreases in all conjugate species by 24 hours post injection.

Example 3 -- Paratope Mapping

Modifications to Ab4 were tested for binding to CD27L by ELISA. CD27L was bound to Maxisorp plates at room temperature for > 2 hours, shaking with 1ug/mL in 100 uL. The plates were then blocked with 250 uL 10% Non-fat dry milk in PBS + 0.05% Tween 20 for 2 hours. After a 4 x 300 uL PBS + 0.05% Tween20 (PBST) wash the modified Ab and purified parental control titrations ranging from 0.045 to 100 ng/mL were applied to the plates and incubated for 1 hour. A horseradish peroxidase conjugated αhuFc detection antibody (Jackson) diluted 1:7000 was applied to the plates after another PBST wash, and incubated for 1 hour. A final wash was performed and TMB substrate was incubated for 10 minutes and stopped with phosphoric acid. An OD reading at 450nm was taken and plotted vs. concentration. These curves were then analyzed with a 3 parameter non linear curve fit and the EC_{50} and Max calculated signal were compared to the purified control. Binding was determined to be similar to parental if the EC_{50} was within 2 fold and the Max signal was greater than 50%. The binding was reduced if the

EC_{50} increased greater than 2 fold and/or the max signal was less than 50%. The binding was abolished if no curve could be generated or the max signal was less than 5%.

Table 8: Light and heavy chain modifications combinations are listed with their expression levels in ug/mL (determined By ForteBio Protein A quantitation) along with their effect on binding.

Combinations	Expression	Binding
N31H-Y58N + N31S-I34M	30.3	Reduce
N31H-Y58N + G33S-I34L	46.7	Abolish
N31H-Y58N + D54E-G55S	9.91	Abolish
N31H-Y58N + S103G-G104S	14.6	Abolish
N31H-Y58N + Parent HC	10.4	Reduce
R24K-S26G + N31S-I34M	13.8	Similar
R24K-S26G + G33S-I34L	16.9	Abolish
R24K-S26G + D54E-G55S	1.87	Reduce
R24K-S26G + S103G-G104S	3.76	Reduce
R24K-S26G + Parent HC	3.43	Similar
L55I-Y58F + N31S-I34M	9.53	Reduce
L55I-Y58F + G33S-I34L	11.6	Abolish
L55I-Y58F + D54E-G55S	1.26	Reduce
L55I-Y58F + S103G-G104S	2.61	Reduce
L55I-Y58F + Parent HC	2.72	Similar
Q95N-T96S + N31S-I34M	24.6	Similar
Q95N-T96S + G33S-I34L	32.7	Abolish
Q95N-T96S + D54E-G55S	1.89	Reduce
Q95N-T96S + S103G-G104S	4.99	Abolish
Q95N-T96S + Parent HC	5.85	Similar
Parent LC + N31S-I34M	31.6	Reduce
Parent LC + G33S-I34L	51.2	Abolish
Parent LC + D54E-G55S	7.03	Reduce
Parent LC + S103G-G104S	10.7	Abolish
Parent LC + Parent HC	9.25	Similar

13 of the 24 modification combinations made to Ab4 retained some level of binding to CD27L. The 9 that did not bind to CD27L had at least one of the following: "G33S-I34L" and "S103G-G104S" heavy chain modifications or the "N31H-Y58N" light chain modification. Of the 97% similar antibodies (2 amino acid modifications) 75% (6 of 8) retained some level of binding, while 56% (7 of 16) of the 94% similar antibodies (4 amino acid modifications) retain some level of binding, 2 of which have similar binding

to the purified parental control. This shows that an antibody as low as 94% similar to Ab4 can still bind CD27L.

SEQUENCE LISTING

SEQ ID NO:1

Human CD27L amino acid sequence

MPEEGSGCSVRRRPYGCVLRAALVPLVAGLVICLVVCIQRFAQAQQQLPLESLG
WDVAELQLNHTGPQQDPRLYWQGGPALGRSFLHGPELDKGQLRIHRDGIYMVH
IQVTLAICSSTTASRHHPTTLAVGICSPASRSISLLRLSFHQGCTIASQRLTPLARGD
TLCTNLTGTLLPSRNTDETFFGVQWVRP

SEQ ID NO:2

Human CD27 precursor amino acid sequence

MARPHPWWLCVLGTLVGLSATPAPKSCPERHYWAQGKLCCQMCEPGTFLVKD
CDQHRKAAQCDPCIPGVSFSPDHHTRPHCESCRHCNSGLLVRNCTITANAECACR
NGWQCRDKECTECDPLPNPSLTARSSQALSPHPQPTHLPYVSEMLEARTAGHMQ
TLADFRQLPARTLSTHWPPQRSLCSSDFIRILVIFSGMFLVFTLAGALFLHQRRKY
RSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP

SEQ ID NO:3

Ab1 Heavy Chain-encoding nucleotide sequence

CAGATGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCCTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGATGGCTCCATCATCAGTGGTGTTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGATACATCTATTACAGTGGGAGCACCTCC
TACAACCCGTCCCTCAAGAGTCGACTTACCATGTCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGCGAGGAGT
GGATACAGCTATGCCCTCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
GCTAGCACCAAGGGCCCATCCGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG
GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG
TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC
TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGA
CCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT
GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG

TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAG
ATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATC
GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG
CTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

SEQ ID NO:4

Ab2 Heavy Chain-encoding nucleotide sequence

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCCTC
ACCTGCACTGTCTCTGGTGACTCCATCATCAGTGGTGGTTACTACTGGAGCTGGATCCGC
CAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTACATCTTTTACAGTGGGAGCACCGAC
TACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAACCAGTTC
TCCCTGAAGCTGAGCTCTGTGACTGCCGCGGACACGGCCGTATATTACTGTGCGAGGAGT
GGATACAGCTATGCCCTCTTTGACCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
GCTAGCACCAAGGGCCCATCCGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG
GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG
TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC
TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGA
CCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT
GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG
TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAG
ATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATC
GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG
CTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

SEQ ID NO:5

Ab4 Heavy Chain-encoding nucleotide sequence

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCGTCTGGATTCACCTTCAGTAACTATGGCATACACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAATAAATACTAT
GCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATGGA
GGATATAGTGGCTACGATTCGGGGTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCAGCTAGCACCAAGGGCCCATCCGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
GTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC
CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTG
GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGG
ACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC
AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAAT
GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGG
GAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT
CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGC
AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

SEQ ID NO:6

Ab5 Heavy Chain-encoding nucleotide sequence

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGATACACCTTCACCAGTTATGATATCAACTGGGTGCGACAGGCC
ACTGGACAAGGGCTTGAGTGGATGGGATGGATGAACCCTAACAGTGGTAACACAGGCTAT
GCACAGAAGTTCCAGGGCAGAGTCACCATGACCAGGAACACCTCCATAAGCACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGGTAC

GATTTTTGGAGTGGTTATTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACC
ACGGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCCGTCTTCCCCCTGGCACCCTCC
TCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCC
GAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCG
GCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC
AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTG
GACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCA
CCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTC
ATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCT
GAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCC
ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
CCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC
TTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC
AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACC
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

SEQ ID NO:7

Ab6 Heavy Chain-encoding nucleotide sequence

CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAGGCTTCTGGTTACACCTTTACCAGCTATGGTATCAGCTGGGTGCGACAGGCC
CCTGGACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTTACACACACTAT
GCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTAC
ATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGACTAC
GGTGGTAACGACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCC
TCAGCTAGCACCAAGGGCCCATCCGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCT
GGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTG
TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCC
TCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAG
CCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACC
CCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAAC

TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGC
AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATC
TCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAG
GAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGAC
ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCC
GTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGG
TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC
ACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

SEQ ID NO:8

Ab7 Heavy Chain-encoding nucleotide sequence

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCGTCTGGATTCACCTTCAGTACCTATGGCATGCACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTAATAAATACTAT
GGAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATAAC
AGTCACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA
GCTAGCACCAAGGGCCCATCCGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGG
GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG
TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC
TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC
AAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGGA
CCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT
GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGG
TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC
AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAG
ATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATC
GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG
CTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
CAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

SEQ ID NO:9

Ab8 Heavy Chain-encoding nucleotide sequence

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATGGTATGATGGAAGTGATAAATACTTT
GCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATGGG
ATAGCAGGAGCTCGCTACGTCTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCAGCTAGCACCAAGGGCCCATCCGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
GTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACC
CAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT
GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTG
GGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGG
ACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC
AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAG
TACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAAT
GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGG
GAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT
CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGC
AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA

SEQ ID NO:10

Ab1 Heavy Chain amino acid sequence

QMQLQESGPGLVKPSQTLSLTCTVSDGSIISGVYYWSWIRQHPGKGLEWIGYIYYSGSTS
YNPSLKSRLTMSVDTSKNQFSLKLSSVTAADTAVYYCARSGYSYALFDYWGQGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE

MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:11

Ab2 Heavy Chain amino acid sequence

QVQLQESGPGLVKPSQTLSLTCTVSGDSIISGGYYWSWIRQHPGKGLEWIGYIFYSGSTD
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARSGYSYALFDHWGQGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:12

Ab4 Heavy Chain amino acid sequence

QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGIHWVRQAPGKGLEWVAVIWYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGGYSGYDSGFDYWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:13

Ab5 Heavy Chain amino acid sequence

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDINWVRQATGQGLEWMGWMNPNSGNTGY
AQKFQGRVTMTRNTSISTAYMELSSLRSEDTAVYYCARGYDFWSGYYYYYYGMDVWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:14

Ab6 Heavy Chain amino acid sequence

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGYTHY
AQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDYGGNDYYGMDVWGQGTTVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:15

Ab7 Heavy Chain amino acid sequence

QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVIWYDGSNKYY
GDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDNSHYYYGMDVWGQGTTVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:16

Ab8 Heavy Chain amino acid sequence

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGSDKYF
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGIAGARYVYFDYWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:17

Ab1 Heavy Chain Variable Domain amino acid sequence

QMQLQESGPGLVKPSQTLSLTCTVSDGSIISGVYYWSWIRQHPGKGLEWIGYIYYSGSTS
YNPSLKSRLTMSVDTSKNQFSLKLSSVTAADTAVYYCARSGYSYALFDYWGQGTLVTVSS

SEQ ID NO:18

Ab2 Heavy Chain Variable Domain amino acid sequence

QVQLQESGPGLVKPSQTLSLTCTVSGDSIISGGYYWSWIRQHPGKGLEWIGYIFYSGSTD
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARSGYSYALFDHWGQGTLVTVSS

SEQ ID NO:19

Ab3 Heavy Chain Variable Domain amino acid sequence

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVISDSGGTTDY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHDYSNRYYFDYWGQGTLVTVSS

SEQ ID NO:20

Ab4 Heavy Chain Variable Domain amino acid sequence

QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGIHWVRQAPGKGLEWVAVIWYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGGYSGYDSGFDYWGQGTLVTV
SS

SEQ ID NO:21

Ab5 Heavy Chain Variable Domain amino acid sequence

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDINWVRQATGQGLEWMGWMNPNSGNTGY
AQKFQGRVTMTRNTSISTAYMELSSLRSEDTAVYYCARGYDFWSGYYYYYYGMDVWGQGT
TVTVSS

SEQ ID NO:22

Ab6 Heavy Chain Variable Domain amino acid sequence

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGYTHY
AQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDYGGNDYYGMDVWGQGTTVTVS
S

SEQ ID NO:23

Ab7 Heavy Chain Variable Domain amino acid sequence

QVQLVESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVIWYDGSNKYY
GDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDNSHYYYGMDVWGQGTTVTVSS

SEQ ID NO:24

Ab8 Heavy Chain Variable Domain amino acid sequence

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDGSDKYF
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGIAGARYVYFDYWGQGTLVTV
SS

SEQ ID NO:25

Ab1 Heavy Chain CDR1 amino acid sequence

SGVYYWS

SEQ ID NO:26

Ab2 Heavy Chain CDR1 amino acid sequence

SGGYYWS

SEQ ID NO:27

Ab3 Heavy Chain CDR1 amino acid sequence

SYAMS

SEQ ID NO:28

Ab4 Heavy Chain CDR1 amino acid sequence

NYGIH

SEQ ID NO:29

Ab5 Heavy Chain CDR1 amino acid sequence

SYDIN

SEQ ID NO:30

Ab6 Heavy Chain CDR1 amino acid sequence
SYGIS

SEQ ID NO:31
Ab7 Heavy Chain CDR1 amino acid sequence
TYGMH

SEQ ID NO:32
Ab8 Heavy Chain CDR1 amino acid sequence
SYGMH

SEQ ID NO:33
Ab1 Heavy Chain CDR2 amino acid sequence
YIYYSGSTSYNPSLKS

SEQ ID NO:34
Ab2 Heavy Chain CDR2 amino acid sequence
YIFYSGSTDYNPSLKS

SEQ ID NO:35
Ab3 Heavy Chain CDR2 amino acid sequence
VISDSGGTTDYADSVKG

SEQ ID NO:36
Ab4 Heavy Chain CDR2 amino acid sequence
VIWYDGSNKYYADSVKG

SEQ ID NO:37
Ab5 Heavy Chain CDR2 amino acid sequence
WMNPNSGNTGYAQKFQG

SEQ ID NO:38
Ab6 Heavy Chain CDR2 amino acid sequence
WISAYNGYTHYAQKLQG

SEQ ID NO:39
Ab7 Heavy Chain CDR2 amino acid sequence
VIWYDGSNKYYGDSVKG

SEQ ID NO:40
Ab8 Heavy Chain CDR2 amino acid sequence
VIWYDGSDKYFADSVKG

SEQ ID NO:41
Ab1 Heavy Chain CDR3 amino acid sequence
SGYSYALFDY

SEQ ID NO:42
Ab2 Heavy Chain CDR3 amino acid sequence
SGYSYALFDH

SEQ ID NO:43
Ab3 Heavy Chain CDR3 amino acid sequence
HDYSNRYYFDY

SEQ ID NO:44
Ab4 Heavy Chain CDR3 amino acid sequence
DGGYSGYDSGFDY

SEQ ID NO:45
Ab5 Heavy Chain CDR3 amino acid sequence
GYDFWSGYYYYYYGMDV

SEQ ID NO:46

Ab6 Heavy Chain CDR3 amino acid sequence

DYGGNDYYGMDV

SEQ ID NO:47

Ab7 Heavy Chain CDR3 amino acid sequence

DNSHYYYGMDV

SEQ ID NO:48

Ab8 Heavy Chain CDR3 amino acid sequence

DGIAGARYVYFDY

SEQ ID NO:49

Ab1 Light Chain-encoding nucleotide sequence

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTTTAGGAGACAGAGTCACC
ATCACTTGCCGGGCAAGTCAGAGCGTTGACAGATATTTCAATTGGTATCAGCAGAAACCT
GGGAAAGCCCCTAAGGTCCTGATCTTTGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCA
AGGTTCGGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCT
GAAGATTTTGCAACTTACTACTGTCAACAGAGCTACAGTACCCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA
TCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTAT
CCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAG
GAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACG
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC
CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:50

Ab2 Light Chain-encoding nucleotide sequence

GACATCCAGATGACCCAGTCCCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCAGTTGCCGGGCAAGTCAGTTCATTGGCAGATATTTCAATTGGTATCAGCAGCAACCA
GGGAAAGCCCCTAAGGTCCTGATCTATGCTGAATCCAGTTTGCAAAGTGGGGTCCCATCA

AGATTCAGTGGCAGTGGATCTGGGACAGAATTCACTCTCACCATCAGCAGTCTGCAACCT
GAAGATTTTGCAAGATACTACTGTCAACAGAGTTACAGTACCCCGTGGACGTTCGGCCAA
GGGACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA
TCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTAT
CCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAG
GAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACG
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC
CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

SEQ ID NO:51

Ab4 Light Chain-encoding nucleotide sequence

GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGAATAGTAATGGATACAACTATTTGGATTGG
TACCTGCAGAAGCCAGGGCAGTCTCCACAGTTCCTGATCTATTTGGGTTCTTATCGGGCC
TCCGGGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAGAATC
AGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGTATACAAACTCTACAAACTCCA
TTCACTTTCGGCCCTGGGACCAAAGTGGATATCAAACGTACGGTGGCTGCACCATCTGTC
TTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTG
CTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAA
TCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
AGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAA
GTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG

SEQ ID NO:52

Ab5 Light Chain-encoding nucleotide sequence

GAAATTGTGTTGACGCAGTCTCCTGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACC
CTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAGA
CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCA
GACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGTCTGGAG
CCTGAAGATTTTGCAGTGTATTACTGTCTGCAGTCTGGTAGCTCTGTCCCGCTCACTTTC
GGCGGAGGGACCAAGGTGGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTC
CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAAC
TTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAAC
TCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACC

CTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCAT
CAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

SEQ ID NO:53

Ab6 Light Chain-encoding nucleotide sequence

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATC
TCTTGTTCTGGAAGCAGCTCCAACATCGGAATTAATTATGTATACTGGTACCAGCAGCTC
CCAGGAACGGCCCCCAAACTCCTCATCTATAGGAGTGATCAGCGGCCCTCAGGGGTCCCT
GACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCCTCAGTGGGCTCCGG
TCCGAGGATGAGGCTGATTATTACTGTGCAGCATGGGATGACAGCCTGAGTGGTGTGGTG
TTCGGCGGAGGGACCAAGCTGACCGTCCTAGGCCAACCGAAAGCGGCGCCCTCGGTCACT
CTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATA
AGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAG
GCGGGAGTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGC
TATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACG
CATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCATAG

SEQ ID NO:54

Ab7 Light Chain-encoding nucleotide sequence

CAGTCTGTGCTGACGCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTCACCATC
TCCTGCACTGGGAGCAGCTCCAACATCGGGGCAGGTTATGATGTAAATTGGTATCAGCAG
TTCCCAGGAACAGCCCCCAAACTCCTCATCTATGTTAACAACAATCGGCCCTCAGGAGTC
CCTGACCGATTCTCTGGCTCCACGTCTGGCACCTCAGCCTCCCTGGCCATCACTGGACTC
CAGGCTGAGGATGAGGCTGATTATTACTGCCAGTCCTATGACACCAGCCTGAGTGCTTCG
GTATTCGGCGGAGGGACCAGACTGACCGTCCTAGGCCAACCGAAAGCGGCGCCCTCGGTC
ACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTC
ATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTC
AAGGCGGGAGTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGC
AGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTC
ACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA

SEQ ID NO:55

Ab8 Light Chain-encoding nucleotide sequence

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACC
ATCACTTGTCGGGCGAGTCAGGGCATTAGCAATTATTTAGCCTGGTTTCAGCAGAAACCA
GGGAAAGCCCCTAAGTCCCTGATCTATGCTGCATCCAGTTTGCAAGGTGGGGTCCCATCA
AAGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGCCT
GAAGATTTTGCAACTTATTACTGCCAACAATATTATAATTACCCATTCACTTTCGGCCCT
GGGACCACAGTGGATATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA
TCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTAT
CCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAG
GAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACG
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC
CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG

SEQ ID NO:56

Ab1 Light Chain amino acid sequence

DIQMTQSPSSLSASLGDRVTITCRASQSVDRYFNWYQQKPGKAPKVLIFAASSLQSGVPS
RFGGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPWTFGQGTKVEVKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:57

Ab2 Light Chain amino acid sequence

DIQMTQSPSSLSASVGDRVTISCRASQFIGRYFNWYQQQPGKAPKVLIYAESSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFARYYCQQSYSTPWTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:58

Ab4 Light Chain amino acid sequence

DIVMTQSPLSLPVTPGEPASISCRSSQSLLNSNGYNYLDWYLQKPGQSPQFLIYLGSYRA
SGVPDRFSGSGSGTDFTLRISRVEAEDVGVYYCIQTLQTPFTFGPGTKVDIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:59

Ab5 Light Chain amino acid sequence

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQRPGQAPRLLIYGASSRATGIP
DRFSGSGSGTDFTLTISSLEPEDFAVYYCLQSGSSVPLTFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:60

Ab6 Light Chain amino acid sequence

QSVLTQPPSASGTPGQRVTISCSGSSSNIGINYVYWYQQLPGTAPKLLIYRSDQRPSGVP
DRFSGSKSGTSASLALSGLRSEDEADYYCAAWDDSLSGVVFGGGTKLTVLGQPKAAPSVT
LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASS
YLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

SEQ ID NO:61

Ab7 Light Chain amino acid sequence

QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVNWYQQFPGTAPKLLIYVNNNRPSGV
PDRFSGSTSGTSASLAITGLQAEDEADYYCQSYDTSLSASVFGGGTRLTVLGQPKAAPSV
TLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAS
SYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

SEQ ID NO:62

Ab8 Light Chain amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQGGVPS
KFSGSGSGTDFTLTISSLQPEDFATYYCQQYYNYPFTFGPGTTVDIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:63

Ab1 Light Chain Variable Domain amino acid sequence

DIQMTQSPSSLSASLGDRVTITCRASQSVDRYFNWYQQKPGKAPKVLIFAASSLQSGVPS
RFGGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPWTFGQGTKVEVK

SEQ ID NO:64

Ab2 Light Chain Variable Domain amino acid sequence

DIQMTQSPSSLSASVGDRVTISCRASQFIGRYFNWYQQQPGKAPKVLIYAESSLQSGVPS
RFSGSGSGTEFTLTISSLQPEDFARYYCQQSYSTPWTFGQGTKVEIK

SEQ ID NO:65

Ab3 Light Chain Variable Domain amino acid sequence

EIVLTQSPGTLSLSPGERATLSCRASQSFSSNYLAWYQQKPGQAPRLFIYGASSRATGIP
DRFSGSGSGTDFTLTISRLEPEDFAVYFCQQYGISPCSFGQGTKLEIK

SEQ ID NO:66

Ab4 Light Chain Variable Domain amino acid sequence

DIVMTQSPLSLPVTPGEPASISCRSSQSLLNSNGYNYLDWYLQKPGQSPQFLIYLGSYRA
SGVPDRFSGSGSGTDFTLRISRVEAEDVGVYYCIQTLQTPFTFGPGTKVDIK

SEQ ID NO:67

Ab5 Light Chain Variable Domain amino acid sequence

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQRPGQAPRLLIYGASSRATGIP
DRFSGSGSGTDFTLTISSLEPEDFAVYYCLQSGSSVPLTFGGGTKVEIK

SEQ ID NO:68

Ab6 Light Chain Variable Domain amino acid sequence

QSVLTQPPSASGTPGQRVTISCSGSSSNIGINYVYWYQQLPGTAPKLLIYRSDQRPSGVP
DRFSGSKSGTSASLALSGLRSEDEADYYCAAWDDSLSGVVFGGGTKLTVL

SEQ ID NO:69

Ab7 Light Chain Variable Domain amino acid sequence

QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVNWYQQFPGTAPKLLIYVNNNRPSGV
PDRFSGSTSGTSASLAITGLQAEDEADYYCQSYDTSLSASVFGGGTRLTVL

SEQ ID NO:70

Ab8 Light Chain Variable Domain amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASSLQGGVPS
KFSGSGSGTDFTLTISSLQPEDFATYYCQQYYNYPFTFGPGTTVDIK

SEQ ID NO:71

Ab1 Light Chain CDR1 amino acid sequence

RASQSVDRYFN

SEQ ID NO:72

Ab2 Light Chain CDR1 amino acid sequence

RASQFIGRYFN

SEQ ID NO:73

Ab3 Light Chain CDR1 amino acid sequence

RASQSFSSNYLA

SEQ ID NO:74

Ab4 Light Chain CDR1 amino acid sequence

RSSQSLLNSNGYNYLD

SEQ ID NO:75

Ab5 Light Chain CDR1 amino acid sequence

RASQSVSSSYLA

SEQ ID NO:76

Ab6 Light Chain CDR1 amino acid sequence

SGSSSNIGINYVY

SEQ ID NO:77

Ab7 Light Chain CDR1 amino acid sequence
TGSSSNIGAGYDVN

SEQ ID NO:78
Ab8 Light Chain CDR1 amino acid sequence
RASQGISNYLA

SEQ ID NO:79
Ab1 Light Chain CDR2 amino acid sequence
AASSLQS

SEQ ID NO:80
Ab2 Light Chain CDR2 amino acid sequence
AESSLQS

SEQ ID NO:81
Ab3 Light Chain CDR2 amino acid sequence
GASSRAT

SEQ ID NO:82
Ab4 Light Chain CDR2 amino acid sequence
LGSYRAS

SEQ ID NO:83
Ab5 Light Chain CDR2 amino acid sequence
GASSRAT

SEQ ID NO:84
Ab6 Light Chain CDR2 amino acid sequence
RSDQRPS

SEQ ID NO:85
Ab7 Light Chain CDR2 amino acid sequence
VNNNRPS

SEQ ID NO:86
Ab8 Light Chain CDR2 amino acid sequence
AASSLQG

SEQ ID NO:87
Ab1 Light Chain CDR3 amino acid sequence
QQSYSTPWT

SEQ ID NO:88
Ab2 Light Chain CDR3 amino acid sequence
QQSYSTPWT

SEQ ID NO:89
Ab3 Light Chain CDR3 amino acid sequence
QQYGISPCS

SEQ ID NO:90
Ab4 Light Chain CDR3 amino acid sequence
IQTLQTPFT

SEQ ID NO:91
Ab5 Light Chain CDR3 amino acid sequence
LQSGSSVPLT

SEQ ID NO:92
Ab6 Light Chain CDR3 amino acid sequence
AAWDDSLSGVV

SEQ ID NO:93

Ab7 Light Chain CDR3 amino acid sequence

QSYDTSLSASV

SEQ ID NO:94

Ab8 Light Chain CDR3 amino acid sequence

QQYYNYPFT

CLAIMS

What is claimed is:

1. A CD27L antigen binding protein comprising:

a) a light chain variable domain having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70;

b) a heavy chain variable domain having at least 90% identity to the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24; or

c) the light chain variable domain of a) and the heavy chain variable domain of b).

2. The CD27L antigen binding protein of claim 1, wherein the light chain variable domain has at least 95% identity to the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70.

3. The CD27L antigen binding protein of claim 1 or 2, wherein the heavy chain variable domain has at least 95% identity to the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24.

4. A CD27L antigen binding protein, comprising:

a) a light chain variable domain having no more than ten amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70;

b) a heavy chain variable domain having no more than ten amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID

NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24; or

c) the light chain variable domain of a) and the heavy chain variable domain of b).

5. The CD27L antigen binding protein of claim 4, wherein the light chain variable domain has no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70.

6. The CD27L antigen binding protein of claim 4 or 5, wherein the heavy chain variable domain has no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24.

7. The CD27L antigen binding protein of claim 1-6, wherein the light chain variable domain comprises the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70.

8. The CD27L antigen binding protein of claim 1-7, wherein the heavy chain variable domain comprises the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24.

9. A CD27L antigen binding protein having a light chain variable domain comprising:

a) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:71; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2

sequence set forth in SEQ ID NO:79; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:87;

b) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:72; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:80; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:88;

c) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:73; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:81; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:89;

d) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:74; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:82; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:90;

e) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:75; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:83; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:91;

f) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:76; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:84; and an LCDR3 having no more than three amino

acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:92;

g) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:77; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:85; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:93; or

h) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:78; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:86; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:94; and

a heavy chain variable domain comprising:

i) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:25; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:33; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:41;

j) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:26; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:34; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:42;

k) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:27; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:35; and an HCDR3 having no more than three amino

acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:43;

l) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:28; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:36; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:44;

m) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:29; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:37; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:45;

n) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:30; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:38; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:46;

o) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:31; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:39; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:47; or

p) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:32; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:40; and an HCDR3 having no more than three amino

acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:48.

10. The CD27L antigen binding protein of claim 9 comprising the light chain variable domain of a) and the heavy chain variable domain of i).

11. The CD27L antigen binding protein of claim 10, wherein the light chain variable domain comprises an LCDR1 as set forth in SEQ ID NO:71; an LCDR2 sequence as set forth in SEQ ID NO:79; and an LCDR3 sequence as set forth in SEQ ID NO:87; and the heavy chain variable domain comprises an HCDR1 as set forth in SEQ ID NO:25; an HCDR2 sequence as set forth in SEQ ID NO:33; and an HCDR3 sequence as set forth in SEQ ID NO:41.

12. The CD27L antigen binding protein of claim 9 comprising the light chain variable domain of b) and the heavy chain variable domain of j).

13. The CD27L antigen binding protein of claim 12, wherein the light chain variable domain comprises an LCDR1 as set forth in SEQ ID NO:72; an LCDR2 sequence as set forth in SEQ ID NO:80; and an LCDR3 sequence as set forth in SEQ ID NO:88; and the heavy chain variable domain comprises an HCDR1 as set forth in SEQ ID NO:26; an HCDR2 sequence as set forth in SEQ ID NO:34; and an HCDR3 sequence as set forth in SEQ ID NO:42.

14. The CD27L antigen binding protein of claim 9 comprising the light chain variable domain of c) and the heavy chain variable domain of k).

15. The CD27L antigen binding protein of claim 14, wherein the light chain variable domain comprises an LCDR1 as set forth in SEQ ID NO:73; an LCDR2 sequence as set forth in SEQ ID NO:81; and an LCDR3 sequence as set forth in SEQ ID NO:89; and the heavy chain variable domain comprises an HCDR1 as set forth in SEQ ID NO:27; an

HCDR2 sequence as set forth in SEQ ID NO:35; and an HCDR3 sequence as set forth in SEQ ID NO:43.

16. The CD27L antigen binding protein of claim 9 comprising the light chain variable domain of d) and the heavy chain variable domain of l).

17. The CD27L antigen binding protein of claim 16, wherein the light chain variable domain comprises an LCDR1 as set forth in SEQ ID NO:74; an LCDR2 sequence as set forth in SEQ ID NO:82; and an LCDR3 sequence as set forth in SEQ ID NO:90; and the heavy chain variable domain comprises an HCDR1 as set forth in SEQ ID NO:28; an HCDR2 sequence as set forth in SEQ ID NO:36; and an HCDR3 sequence as set forth in SEQ ID NO:44.

18. The CD27L antigen binding protein of claim 9 comprising the light chain variable domain of e) and the heavy chain variable domain of m).

19. The CD27L antigen binding protein of claim 18, wherein the light chain variable domain comprises an LCDR1 as set forth in SEQ ID NO:75; an LCDR2 sequence as set forth in SEQ ID NO:83; and an LCDR3 sequence as set forth in SEQ ID NO:91; and the heavy chain variable domain comprises an HCDR1 as set forth in SEQ ID NO:29; an HCDR2 sequence as set forth in SEQ ID NO:37; and an HCDR3 sequence as set forth in SEQ ID NO:45.

20. The CD27L antigen binding protein of claim 9 comprising the light chain variable domain of f) and the heavy chain variable domain of n).

21. The CD27L antigen binding protein of claim 20, wherein the light chain variable domain comprises an LCDR1 as set forth in SEQ ID NO:76; an LCDR2 sequence as set forth in SEQ ID NO:84; and an LCDR3 sequence as set forth in SEQ ID NO:92; and the heavy chain variable domain comprises an HCDR1 as set forth in SEQ ID NO:30; an

HCDR2 sequence as set forth in SEQ ID NO:38; and an HCDR3 sequence as set forth in SEQ ID NO:46.

22. The CD27L antigen binding protein of claim 9 comprising the light chain variable domain of g) and the heavy chain variable domain of o).

23. The CD27L antigen binding protein of claim 22, wherein the light chain variable domain comprises an LCDR1 as set forth in SEQ ID NO:77; an LCDR2 sequence as set forth in SEQ ID NO:85; and an LCDR3 sequence as set forth in SEQ ID NO:93; and the heavy chain variable domain comprises an HCDR1 as set forth in SEQ ID NO:31; an HCDR2 sequence as set forth in SEQ ID NO:39; and an HCDR3 sequence as set forth in SEQ ID NO:47.

24. The CD27L antigen binding protein of claim 9 comprising the light chain variable domain of h) and the heavy chain variable domain of p).

25. The CD27L antigen binding protein of claim 24, wherein the light chain variable domain comprises an LCDR1 as set forth in SEQ ID NO:78; an LCDR2 sequence as set forth in SEQ ID NO:86; and an LCDR3 sequence as set forth in SEQ ID NO:94; and the heavy chain variable domain comprises an HCDR1 as set forth in SEQ ID NO:32; an HCDR2 sequence as set forth in SEQ ID NO:40; and an HCDR3 sequence as set forth in SEQ ID NO:48.

26. The CD27L antigen binding protein of any of claims 1-25, wherein the antigen binding protein specifically binds human CD27L with an affinity of less than or equal to 2×10^{-11} M.

27. The CD27L antigen binding protein of any of claims 1-26, wherein the antigen binding protein inhibits binding of CD27L to CD27.

28. The CD27L antigen binding protein of any of claims 1-27, wherein the antigen binding protein is an antibody.

29. The CD27L antigen binding protein of claim 28, wherein the antibody is a human antibody.

30. The CD27L antigen binding protein of claim 29 comprising a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID:56 and the heavy chain comprises the amino acid sequence set forth in SEQ ID NO:10.

31. The CD27L antigen binding protein of claim 29 comprising a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID:57 and the heavy chain comprises the amino acid sequence set forth in SEQ ID NO:11.

32. The CD27L antigen binding protein of claim 29 comprising a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID:58 and the heavy chain comprises the amino acid sequence set forth in SEQ ID NO:12.

33. The CD27L antigen binding protein of claim 29 comprising a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID:59 and the heavy chain comprises the amino acid sequence set forth in SEQ ID NO:13.

34. The CD27L antigen binding protein of claim 29 comprising a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID:60 and the heavy chain comprises the amino acid sequence set forth in SEQ ID NO:14.

35. The CD27L antigen binding protein of claim 29 comprising a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID:61 and the heavy chain comprises the amino acid sequence set forth in SEQ ID NO:15.

36. The CD27L antigen binding protein of claim 29 comprising a light chain and a heavy chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID:62 and the heavy chain comprises the amino acid sequence set forth in SEQ ID NO:16.

37. The CD27L antigen binding protein of any of claims 1-36, wherein the CD27L antigen binding protein is conjugated to a chemotherapeutic agent.

38. The CD27L antigen binding protein of claim 37, wherein a linker conjugates the chemotherapeutic agent to the CD27L antigen binding protein.

39. The CD27L antigen binding protein of claim 38, wherein the linker is a non-cleavable linker.

40. The CD27L antigen binding protein of claim 39, wherein the linker comprises MCC.

41. The CD27L antigen binding protein of any of claims 38-40, wherein the chemotherapeutic agent is conjugated to one or more lysines contained with a polypeptide of the CD27L antigen binding protein.

42. The CD27L antigen binding protein of any of claims 37-41, wherein the chemotherapeutic agent is DM1.

43. A composition of CD27L antigen binding proteins of claim 42, wherein the average number of DM1 molecules per CD27L antigen binding protein is between 1 and 10.

44. The composition of CD27L antigen binding proteins of claim 43, wherein the average number of DM1 molecules per CD27L antigen binding protein is between 3 and 7.

45. The composition of CD27L antigen binding proteins of claim 44, wherein the average number of DM1 molecules per CD27L antigen binding protein is between 4 and 6.

46. The composition of CD27L antigen binding proteins of claim 44, wherein the average number of DM1 molecules per CD27L antigen binding protein is about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, or about 6.0.

47. The composition of CD27L antigen binding proteins of any of claims 43-46, wherein the composition is a pharmaceutical composition comprising a therapeutically effective amount of the CD27L antigen binding protein.

48. The composition of CD27L antigen binding proteins of claim 47, wherein the pharmaceutical composition is lyophilized.

49. An isolated nucleic acid encoding a polypeptide, said polypeptide comprising:

a) a light chain variable domain having at least 95% identity to the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70;

b) a heavy chain variable domain having at least 95% identity to the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24;

c) a light chain variable domain having no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, or SEQ ID NO:70;

d) a heavy chain variable domain having no more than five amino acid additions, deletions or substitutions from the amino acid sequence set forth in SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:24;

e) a light chain variable domain comprising:

i) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:71; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:79; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:87;

ii) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:72; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:80; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:88;

iii) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:73; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:81; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:89;

iv) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:74; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:82; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:90;

v) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:75; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:83; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:91;

vi) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:76; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:84; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:92;

vii) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:77; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:85; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:93; or

viii) an LCDR1 having no more than three amino acid additions, deletions, or substitutions from the LCDR1 sequence set forth in SEQ ID NO:78; an LCDR2 having no more than three amino acid additions, deletions, or substitutions from the LCDR2 sequence set forth in SEQ ID NO:86; and an LCDR3 having no more than three amino acid additions, deletions, or substitutions from the LCDR3 sequence set forth in SEQ ID NO:94; or

f) a heavy chain variable domain comprising:

i) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:25; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:33; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:41;

ii) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:26; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:34; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:42;

iii) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:27; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:35; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:43;

iv) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:28; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:36; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:44;

v) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:29; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:37; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:45;

vi) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:30; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:38; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:46;

vii) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:31; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:39; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:47; or

viii) an HCDR1 having no more than three amino acid additions, deletions, or substitutions from the HCDR1 sequence set forth in SEQ ID NO:32; an HCDR2 having no more than three amino acid additions, deletions, or substitutions from the HCDR2 sequence set forth in SEQ ID NO:40; and an HCDR3 having no more than three amino acid additions, deletions, or substitutions from the HCDR3 sequence set forth in SEQ ID NO:48.

50. The isolated nucleic acid of claim 49, wherein the polypeptide comprises a antibody light chain.

51. The isolated nucleic acid of claim 50, wherein the light chain is encoded by a nucleic acid comprising a nucleotide sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, or SEQ ID NO:55.

52. The isolated nucleic acid of claim 51, wherein the light chain is encoded by a nucleic acid comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, or SEQ ID NO:55.

53. The isolated nucleic acid of claim 52, wherein the light chain is encoded by a nucleic acid comprising a nucleotide sequence at least 95% identical to the nucleotide sequence set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, or SEQ ID NO:55.

54. The isolated nucleic acid of claim 53, wherein the light chain is encoded by a nucleic acid comprising a nucleotide sequence set forth in SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, or SEQ ID NO:55.

55. The isolated nucleic acid of claim 49, wherein the polypeptide comprises a antibody heavy chain.

56. The isolated nucleic acid of claim 55, wherein the heavy chain is encoded by a nucleic acid comprising a nucleotide sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9.

57. The isolated nucleic acid of claim 56, wherein the heavy chain is encoded by a nucleic acid comprising a nucleotide sequence at least 90% identical to the nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9.

58. The isolated nucleic acid of claim 57, wherein the heavy chain is encoded by a nucleic acid comprising a nucleotide sequence at least 95% identical to the nucleotide sequence set forth in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:9.

59. The isolated nucleic acid of claim 58, wherein the heavy chain is encoded by a nucleic acid comprising a nucleotide sequence set forth in SEQ ID NO:49, SEQ ID

NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, or SEQ ID NO:55.

60. An expression vector comprising the isolated nucleic acid of any of claims 49-59.

61. The expression vector of claim 60, wherein the isolated nucleic acid encodes an antibody light chain.

62. The expression vector of claim 60, wherein the isolated nucleic acid encodes an antibody heavy chain.

63. The expression vector of claim 61, further comprising an isolated nucleic acid encoding an antibody heavy chain.

64. A recombinant host cell comprising an isolated nucleic acid of any of claims 49-59 operably linked to a promoter.

65. A recombinant host cell comprising an expression vector of claim 61 or 62.

66. The recombinant host cell of claim 65, wherein the host cell comprises an expression vector of claim 61 and 62.

67. The recombinant host cell of claim 66, wherein the host cell secretes an antibody that binds CD27L.

68. The recombinant host cell of any of claims 64-67, wherein the cell is of mammalian origin.

69. The recombinant host cell of claim 68, wherein the cell is a Chinese hamster ovary (CHO) cell line.

70. A method of making a CD27L antibody drug conjugate, said method comprising the steps of:

a) providing a CD27L antigen binding protein of any one of claims 1-36;

b) conjugated the CD27L antigen binding protein to a linker; and

c) conjugating a drug to said linker.

71. A method of making a CD27L antibody drug conjugate, said method comprising the steps of:

a) providing a CD27L antigen binding protein of any one of claims 1-36; and

b) conjugating a linker covalently bound to a drug to said CD27L antigen binding protein.

72. The method of claim 70 or 71, wherein the CD27L antigen binding protein is an antibody.

73. The method of claim 72, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:63 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:17.

74. The method of claim 73, wherein the antibody is Ab1.

75. The method of claim 72, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:64 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:18.

76. The method of claim 75, wherein the antibody is Ab2.

77. The method of claim 72, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:65 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:19.

78. The method of claim 77, wherein the antibody is Ab3.

79. The method of claim 72, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:66 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:20.

80. The method of claim 79, wherein the antibody is Ab4.

81. The method of claim 72, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:67 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:21.

82. The method of claim 81, wherein the antibody is Ab5.

83. The method of claim 72, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:68 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:22.

84. The method of claim 83, wherein the antibody is Ab6.

85. The method of claim 72, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:69 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:23.

86. The method of claim 85, wherein the antibody is Ab7.

87. The method of claim 72, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:70 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:24.

88. The method of claim 87, wherein the antibody is Ab8.

89. The method of any one of claims 70-88, wherein the linker comprises MCC.

90. The method of any one of claims 70-89, wherein the drug comprises DM1.

91. A method of treating cancer, said method comprising administering a therapeutically effective amount of a CD27L antigen binding protein of any one of claims 1-36 to a patient in need thereof.

92. The method of claim 91, wherein the CD27L antigen binding protein is an antibody.

93. The method of claim 92, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:63 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:17.

94. The method of claim 93, wherein the antibody is Ab1.

95. The method of claim 92, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:64 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:18.

96. The method of claim 95, wherein the antibody is Ab2.

97. The method of claim 92, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:65 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:19.

98. The method of claim 97, wherein the antibody is Ab3.

99. The method of claim 92, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:66 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:20.

100. The method of claim 99, wherein the antibody is Ab4.

101. The method of claim 92, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:67 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:21.

102. The method of claim 101, wherein the antibody is Ab5.

103. The method of claim 92, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:68 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:22.

104. The method of claim 103, wherein the antibody is Ab6.

105. The method of claim 92, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:69 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:23.

106. The method of claim 105, wherein the antibody is Ab7.

107. The method of claim 92, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:70 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:24.

108. The method of claim 107, wherein the antibody is Ab8.

109. The method of any one of claims 92-108, wherein a pharmaceutical composition comprises the antibody.

110. The method of claim 109, wherein the antibody comprises enchanced effector function.

111. The method of claim 109, wherein the pharmaceutical composition comprises a population of antibody drug conjugates.

112. The method of claim 111, wherein the antibody drug conjugate comprises an MCC linker.

113. The method of claim 111 or 112, wherein the antibody drug conjugate comprises DM1.

114. The method of claim 113, wherein the average number of DM1 molecules per antibody is between 1 and 10.

115. The method of claim 114, wherein the average number of DM1 molecules per antibody is between 3 and 7.

116. The method of claim 115, wherein the average number of DM1 molecules per antibody is between 4 and 6.

117. The method of claim 116, wherein the average number of DM1 molecules per antibody is about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, or about 6.0.

118. The method of any of claims 91--117, wherein a sample from the patient is tested for CD27L expression.

119. The method of claim 118, wherein the sample is tested for CD27L mRNA expression.

120. The method of claim 118, wherein the sample is tested for CD27L protein expression.

121. The method of any one of claims 118-120, wherein the sample is a blood sample.

122. The method of any one of claims 118-120, wherein the sample is a biopsy.

123. The method of any one of claims 91-122, wherein the cancer is renal cell carcinomas (RCC), clear cell RCC, head and neck cancer, glioblastoma, breast cancer, brain tumor, nasopharangeal carcinoma, non-Hodgkin's lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), Burkitt's lymphoma, anaplastic large-cell lymphomas (ALCL), multiple myeloma, cutaneous T-cell lymphomas, nodular small cleaved-cell lymphomas, lymphocytic lymphomas, peripheral T-cell lymphomas, Lennert's lymphomas, immunoblastic lymphoma, T-cell leukemia/lymphomas (ATLL), adult T-cell leukemia (T-ALL), entroblastic/centrocytic (cb/cc) follicular lymphoma cancer, diffuse large cell lymphoma of B lineage, angioimmunoblastic lymphadenopathy (AILD)-like T cell lymphoma, HIV associated body cavity based lymphoma, embryonal carcinoma, undifferentiated carcinoma of the rhino-pharynx, Castleman's disease, Kaposi's Sarcoma, multiple myeloma, Waldenstrom's macroglobulinemia or other B-cell lymphoma.

124. The method of claim 123, wherein the cancer is RCC.

125. The method of claim 123, wherein the cancer is NHL.

126. The method of claim 123, wherein the cancer is CLL.

127. A method of treating an autoimmune or inflammatory disorder said method comprising administering a therapeutically effective amount of a CD27L antigen binding protein of any one of claims 1-36 to a patient in need thereof.

128. The method of claim 127, wherein the CD27L antigen binding protein is an antibody.

129. The method of claim 128, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:63 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:17.

130. The method of claim 129, wherein the antibody is Ab1.

131. The method of claim 128, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:64 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:18.

132. The method of claim 131, wherein the antibody is Ab2.

133. The method of claim 128, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:65 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:19.

134. The method of claim 133, wherein the antibody is Ab3.

135. The method of claim 128, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:66 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:20.

136. The method of claim 135, wherein the antibody is Ab4.

137. The method of claim 128, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:67 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:21.

138. The method of claim 137, wherein the antibody is Ab5.

139. The method of claim 128, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:68 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:22.

140. The method of claim 139, wherein the antibody is Ab6.

141. The method of claim 128, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:69 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:23.

142. The method of claim 141, wherein the antibody is Ab7.

143. The method of claim 128, wherein the antibody comprises a light chain variable domain amino acid sequence as set forth in SEQ ID NO:70 and a heavy chain variable domain amino acid sequence as set forth in SEQ ID NO:24.

144. The method of claim 143, wherein the antibody is Ab8.

145. The method of claim 127, wherein the antigen binding protein inhibits binding of CD27 to CD27L.

146. The method of any one of claims 127-145, wherein the autoimmune or inflammatory disorder is systemic lupus erythematosus (SLE), insulin dependent diabetes mellitus (IDDM), inflammatory bowel disease (IBD), multiple sclerosis (MS), psoriasis, autoimmune thyroiditis, rheumatoid arthritis (RA), or glomerulonephritis.

147. The method of any of claims 127-145, wherein the treatment inhibits or prevents transplant rejection in the patient.

148. The method of any of claims 127-145, wherein the treatment inhibits or prevents graft versus host disease (GVHD).